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From the Editors' Desk

Dear Medical Arthropodologists &
Public Health Scientists,

> "*Nature is the source of all true knowledge. She has her own logic, her own laws, she has no effect without cause nor invention without necessity*."

We are back again, on time, with a new set of research publications based on a variety of subjects so important to public health managers and policy makers. These papers, however, represent only a fraction of the vast and unexplored spectrum of disciplines within the unfathomable depths of the integrated science of medical arthropodology and public health which are to gradually and periodically come to surface in the future issues, in a way so as to generate irrefutable scientific knowledge toward the varied and diverse world of entrepreneurship! This also implies that tides are changing; from theoretical to practical medical arthropodology! Thus, we are endeavouring hard to publish on the pages of *Journal of Medical Arthropodology & Public Health* – a broad-scope, open access-cum-print journal publishing basic and applied research that has a positive impact on translation of sophisticated data-based scientific studies into usable products by the end-user – the research papers that truly serve the science and society.

Our aim at *Journal of Medical Arthropodology & Public Health* is to maximize the global visibility and impact of your published articles. The *Journal of Medical Arthropodology & Public Health* is for all those men and women who are interested in scientific discovery, and in its industrial, commercial and social consequences. It

will report, explore and interpret the results of human endeavour set in the context of science and society. Through *Journal of Medical Arthropodology & Public Health* scientists will be motivated to think beyond their discipline and believe that collaborative science and interdisciplinary ideas can advance national policies related to the control of vector-borne diseases, on one hand, and bring other biomedical concerns under thorough scanning and surveillance, on the other, to inspire new thinking. This is the time of Artificial Intelligence which has enabled bridging consilience, on one hand, and vastly elevated the element of polyintelligence in medical arthropodology, on the other. Thus, in this issue, our authors describe some of the challenges in controlling the various vector-borne & zoonotic diseases, besides exploring new horizons in the biology of medically important arthropods and pave pathways to consolidate new ideas toward their control. The issue, as always before, additionally offers its readers with fresh food for thoughts under the *Perspective Section* and an uncustomary, and yet innovative, motivational and elegantly archivable *Scientists' Biobibliography* Section which are unique in the world of scientific journals. Your satisfaction is our gain.

"*Knowing is not enough; we must apply.*
Willing is not enough; we must do."

Soliciting your continued support and patronage in our comprehensive evolution together both as the journal and the authors, we remain, as heretofore,

Yours cordially,

Prof. Dr B.K. Tyagi & Dr Rina Tilak

December 1, 2024 Chief Editor Executive Editor

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Perspective

'SPIRITUALITY' - A *FORTIORI* FOURTH DIMENSION OF HUMAN HEALTH: EMPHASIS ON A TIME TESTED PRACTICE IN INDIA

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"पहला सुख निरोगी काया", implying, "Healthy Body - The True Happiness", is a time tested adage which the whole world unanimously understand and acknowledge. In our childhood we have read Billy Graham's famous rhyme: "*When wealth is lost, nothing is lost; when health is lost, something is lost; and when character is lost, all is lost.*" There is hardly any skepticism that it is 'Character' which is the most valued human quality during most part of life, albeit the fact that it is 'Health' which accompanies the worldly odyssey lifelong! Therefore, health is the most important factor in life not only for mere existence but for living with all faculties of function in good state justifying the maxim: "*A good mind lives only in a healthy body*." Mahatma Gandhi, 'The Father of the Nation', advocated, "*It is health that is real wealth and not*

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pieces of gold and silver." According to World Health Organization (WHO), health is defined as "*a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.*" This interpretation of health was adopted by the WHO's constitution laid down in 1946 and has not been amended despite criticism from many different disciplinary backgrounds. Apparently, while physical and social factors are easily discernible and/or measureable as well as factorable, mental health is more than just the absence of mental disorders or disabilities and needs more critical evaluation. Indian medical scriptures of yore, like '*Atharva Ved'*, written 400 BC, "*Vedic Health enlivens the inner intelligence of the body and thereby maintains the vitality of the physiology*…", hinting on a new fundamental principle invariably associated with health, which I am calling "Spirituality – the fourth dimension" of health.

Spirituality needs not be confused with either mental or psychological factors of health, although they are an integral and essential component of health. Clearly enough the latter is distinguishably a state of well-being in which an individual realizes his or her own abilities, can cope with the various challenges of life, can work productively and is able to contribute to his or her community. It is also true that mental health is fundamental to our collective and individual ability as humans to think, emote, interact with each other, earn a living and conduct life in harmony with each other. This is why that on this basis alone, the promotion, protection and restoration of mental health can be regarded as a vital concern of individuals, communities and societies throughout the world. In essence, according to the WHO's concept, there is no health without mental health, determined by a range of socioeconomic, biological and environmental factors achievable largely through cost-effective public health and intersectoral strategies and interventions. In my lifelong experiments with 'Spirituality', I have eventually regarded this to be the supreme factor or power on self-health.

Likewise the three well-popularized dimensions of health; physical, mental and social, 'spirituality', too, is demonstrable, repeatable and factorable without a single instance of vicissitude. Spirituality can be taught, practiced and disseminated through oral and written routes of communication. Unlike mental dimension, spirituality is indiscernible, though palpable and absorbable. Spirituality is a deep-rooted science which the science *per se* has continuously been struggling to define and come up at par with. Spirituality is a broad

concept that involves a sense of connection to something greater than oneself, and how that connection provides meaning to life. It can also include a person's sense of peace, purpose, and connection to others such as, for example, the Nature. When practiced devotedly spirituality can improve quality of life; patients with advanced cancer who found comfort in their spirituality were happier and had less pain. Spirituality can help people cope with illness, pain, and life stresses. It can reduce health-risk behaviors and is associated with higher levels of health-related behaviors. In essence, the 'inner behavior' of self is considered supreme for attaining a holistic health status. Thus, spirituality is an essential part of modern healthcare and can be integrated into patient care.

Spiritual wellness is not a new concept and regarded as an important part of overall health and well-being, but bringing it to an international forum of discussion is certainly inevitablyunequivocally for the first time. More so, spirituality has its cradle in India - *Bharat* where it is still practiced through '*Yog*' and meditation (Tyagi, 2024) as a pathway to attain the ultimate state of health.

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Commentary

LYME BORRELIOSIS IN INDIA: GROWING CONCERN AMID CLIMATE CHANGE

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ABSTRACT

Vector-borne diseases, particularly those spread by ticks, are a significant global health threat. Lyme borreliosis, caused by *Borrelia burgdorferi sensu lato* complex and transmitted primarily by black-legged ticks (*Ixodes* species) which transmits the bacteria from small mammals especially white footed mice and some migratory birds which serve as reservoirs, is a rising concern in India, although, historically, rare due to its tropical climate. Emerging evidence links climate change to the expansion of tick populations, particularly in northern India, where environmental conditions increasingly mirror those in Lyme-endemic regions. Despite reports of Lyme borreliosis cases across many states, the disease is likely underreported due to limited awareness, diagnostic challenges, and a lack of robust surveillance systems. The overlapping symptoms with other febrile

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illnesses, such as malaria and dengue, further complicate diagnosis. There is a need to explore the growing risk of Lyme borreliosis in India, emphasizing the need for enhanced surveillance, better diagnostic tools, and improved public and healthcare provider awareness. The role of climate change in expanding tick habitats and its potential to increase Lyme borreliosis transmission is discussed, along with preventive measures such as public health campaigns and tick habitat management. Developing epidemiological and laboratory capacity, particularly in regions experiencing rising tick populations, is essential for early detection and control. Addressing the root causes of climate change and implementing data-driven, evidence-based strategies are crucial in mitigating the long-term risk of Lyme borreliosis in India. Strengthening healthcare systems and integrating climate adaptation measures will be key to managing this growing public health threat.

Keywords: Lyme, Borreliosis, Climate Change, India

INTRODUCTION

Vector-borne diseases account for a significant portion of the global infectious disease burden, particularly in tropical and subtropical regions, causing over 700,000 deaths annually[1]. Tick-borne diseases, including Lyme borreliosis, Crimean-Congo Haemorrhagic Fever, Ehrlichiosis, Indian Tick Typhus, Anaplasmosis, Tularemia, Babesiosis, and Kyasanur Forest Disease, are on the rise and contribute to substantial morbidity and mortality in humans[2]. Despite their widespread impact, gaps in epidemiology and surveillance hinder effective control, making Tick-born diseases an increasingly critical public health concern. Climate change plays a significant role in the transmission and spread of tick-borne diseases, as it directly impacts tick populations, their habitats, and the behaviour of the animals that serve as hosts for ticks[3]. Lyme borreliosis has been recognized as a significant public health issue, especially in North America and Europe however, in recent years, it is emerging as a growing concern in India due to various ecological, environmental, and climatic factors[4]. This article delves into the growing concern of Lyme borreliosis in India, focusing on how climate change may be exacerbating its spread, the challenges in its diagnosis and management, and the strategies that can be employed to mitigate this threat.

Epidemiology of Lyme borreliosis

Lyme borreliosis is caused by the bacterium *Borrelia burgdorferi sensu lato* complex and transmitted primarily by black-legged ticks (*Ixodes scapularis* & *Ixodes ricinus* species) which transmits the bacteria from small mammals especially white footed mice and some migratory birds which serve as reservoirs[5]. India has not usually been regarded as a hotspot for Lyme borreliosis due to its tropical climate, which is generally considered less favourable for the survival of Ixodes ticks. Nonetheless, cases of Lyme borreliosis has been reported from few states of India, viz. Himachal Pradesh, Northeastern states, Sikkim, Karnataka, Kerala and Haryana[6-8]. Although, the actual number of confirmed Lyme borreliosis cases in India remains unknown, it is suspected that the disease is underreported. This underreporting is attributed to limited awareness, diagnostic difficulties, and the lack of a robust surveillance system for tick-borne diseases. A closer look at seroprevalence studies on Lyme borreliosis in various states of India, in absence of a database or reporting system on disease burden, varied from 2% to 17%[9-11]. The detection of Ixodes ticks, the vectors for *Borrelia burgdorferi*, in some northern areas, particularly those with temperate climates, mirrors the environmental conditions of regions where Lyme borreliosis is endemic. These endemic regions tend to have high humidity, abundant vegetation, and the presence of both tick vectors and reservoir hosts, particularly small mammals like rodents and deer. Environmental and ecological factors such as temperature, humidity, and land use significantly influence the distribution and transmission of Lyme disease[12-15].

Challenges

The rise of Lyme borreliosis in India poses several challenges for healthcare providers and public health authorities, particularly in terms of diagnosis, treatment, and surveillance. One of the primary challenges in diagnosing Lyme borreliosis in India is the lack of awareness among both healthcare professionals and the general public. The overlapping symptoms of Lyme borreliosis with other febrile illnesses, like malaria, dengue, and chikungunya, further complicates its diagnosis. As a result, many cases go undiagnosed or are misdiagnosed, leading to delayed treatment and increased risk of complications. Healthcare providers in India need to be educated that Lyme disease can have varied presentations including fever, fatigue, joint pain, and skin rash, as well as more serious joint and nervous system

complications and should maintain a high index of suspicion, especially in regions where tick populations are increasing. Public health campaigns that raise awareness about the risk of tick bites and the importance of early diagnosis can also help reduce the burden of the disease. Accurate diagnosis of Lyme borreliosis relies on a combination of clinical evaluation and laboratory testing. However, laboratory testing for Lyme borreliosis is not widely available in India, and many healthcare facilities lack the necessary resources and expertise to diagnose the disease accurately. Serological tests, such as enzyme-linked immunosorbent assay (ELISA) and Western blot, are commonly used to detect antibodies against *Borrelia burgdorferi*[16]. However, these tests may produce false-negative results in the early stages of infection when antibodies have not yet developed[17]. Polymerase chain reaction (PCR) testing, which detects the presence of Borrelia DNA, is more accurate but less accessible in resource-limited settings.

The standard treatment for Lyme borreliosis is a course of antibiotics, such as doxycycline, amoxicillin, or cefuroxime, which is effective in most cases if administered early. However, in India, there is a risk of under-treatment or inappropriate treatment due to the lack of familiarity with the disease among healthcare providers. In addition, some patients may develop post-treatment Lyme disease syndrome (PTLDS), which can cause persistent symptoms even after antibiotic treatment[18]. To address these challenges, it is important to establish clear treatment guidelines for Lyme borreliosis in India and to ensure that healthcare providers have access to the necessary medications. Early diagnosis and prompt treatment are critical in preventing the progression of the disease to its later stages, where complications such as arthritis and neurological issues can develop.

Currently, India lacks comprehensive surveillance systems for tick-borne diseases, and the true incidence of Lyme borreliosis in the country is likely underestimated. Strengthening our surveillance system as in USA & Canada where expansion and encroachment of tick vector species & other disease transmission factors are effectively monitored, particularly in regions where tick populations are expanding, can help identify trends in Lyme borreliosis transmission and guide public health interventions. Collaboration between healthcare providers, researchers, and public health authorities is essential for collecting accurate data on the prevalence of Lyme borreliosis in India and for developing strategies to mitigate its impact.

Climate change and Lyme borreliosis in India

India is highly vulnerable to the impacts of climate change, with its diverse geography and large population intensifying the challenges posed by rising global temperatures. The country has witnessed increasingly erratic weather patterns, including more frequent heat waves, intense rainfall, and severe flooding. For instance, the temperatures were consistently 3-8°C above normal, breaking many decadal and some all-time records in several parts of the country. Many parts of the country experienced extreme weather events like extremely heavy rainfall, floods, landslide, lightning, thunderstorm, etc. (IMD Annual report, 2022)[19]. Global warming and habitat changes have contributed to an increase in tick populations, including *Ixodes scapularis*, *Amblyomma americanum*, *Rhipicephalus sanguineus*, and *Dermacentor variabilis* which have been extensively reported in the USA and Canada[12,15,20-23]. It is also well recorded that temperature and humidity not only impact tick biology but also the development of Borrelia in ticks. The optimal temperature range for both tick activity and Borrelia development is between 10°C and 25°C. Below 4°C, tick activity slows down significantly, and above 30°C, desiccation becomes a major risk. High relative humidity (RH), ideally above 85%, is critical for tick survival and development and indirectly for the persistence of Borrelia[24-27]. A spatio-temporal increase in Lyme borreliosis is likely associated with climate change, which favours tick survival and the spread of Borrelia[28-30]. Climate change is leading to an increase in the length of tick activity seasons, allowing ticks to feed on hosts, reproduce, and spread borreliosis for extended periods each year. Regions that were once too cold for ticks are now becoming suitable habitats due to rising temperatures. In India, the northern regions, such as Himachal Pradesh, Jammu and Kashmir, and Uttarakhand, are experiencing shifts in climate patterns, with warmer temperatures creating a more hospitable environment for ticks. As a result, these areas are seeing an increase in tick populations, raising the risk of Lyme borreliosis transmission. Additionally, changes in monsoon patterns and the reduction of snowfall in higher altitudes are contributing to changes in vegetation and tick habitats. The relationship between temperature, relative humidity, and Lyme borreliosis is complex, but overall, warmer and more humid conditions seem to favour the tick vectors and their associated pathogens, thereby increasing the risk and spread of Lyme disease[31-32].

Ticks require hosts, such as rodents, deer and birds, to feed on and complete their life cycles. Climate change is influencing the migration and population dynamics of these animals, leading to changes in tick-host interactions[33]. Warmer temperatures and changes in vegetation may lead to increased interactions between ticks and their hosts, facilitating the spread of *Borrelia burgdorferi*. In India, climate change is affecting the behaviour and distribution of animals that serve as tick hosts. For example, the expansion of forested areas and changes in wildlife migration patterns can increase the likelihood of humans coming into contact with ticks, especially in rural and forested regions. This is particularly concerning for individuals living in or near wildlife habitats, where they may be more susceptible to tick bites. Climate change is also influencing human behaviour and land use patterns, which in turn affect tick exposure[34]. As temperatures rise and ecosystems shift, people are more likely to spend time outdoors, increasing their risk of tick bites. Additionally, deforestation, urbanization, and agricultural expansion can disrupt natural ecosystems, leading to changes in tick populations and the spread of tick-borne diseases. In India, rapid urbanization and changes in land use have led to the encroachment of human settlements into forested areas, where ticks are more prevalent. This increases the likelihood of tick-human interactions, particularly in regions where agriculture, forestry, and tourism are common.

Given the growing concern about Lyme borreliosis in India amid climate change, it is crucial to implement preventive measures to reduce the risk of tick bites and the spread of Lyme borreliosis. Raising awareness about Lyme borreliosis and the importance of tick bite prevention is a key strategy for reducing the risk of infection. Public health campaigns can educate individuals about the proper use of insect repellents, wearing protective clothing, and conducting regular tick checks after spending time outdoors. These campaigns should target both rural and urban populations, particularly in regions where tick populations are on the rise. Controlling tick populations by managing tick habitats is an effective strategy for reducing the risk of Lyme borreliosis transmission. Measures such as clearing tall grass, removing leaf litter, and keeping lawns trimmed can reduce the number of ticks in residential areas. In forested or rural regions, creating buffer zones between residential areas and tick habitats can further minimize the risk of tick bites. Additionally, the use of acaricides (tick-killing chemicals) in areas with high tick populations can help reduce tick density[35]. However, the use of chemical treatments should be carefully monitored to avoid environmental harm. Individuals can take

several personal protective measures to reduce their risk of tick bites. These include wearing long sleeves shirt, long pants, and closed-toe shoes when spending time outdoors, especially in tick-prone areas[4]. Applying repellents to exposed skin and clothing; conducting thorough tick checks after spending time outdoors and removing any attached ticks promptly using fine-tipped tweezers coupled with bathing or showering within two hours of being outdoors will decidedly reduce the likelihood of tick attachment.

Way Forward

A data-driven, evidence-based approach is key to drafting effective policies against Lyme borreliosis and other emerging tick-borne illnesses, while a robust surveillance system and focused attention from health systems and policymakers are essential for effective management. Establishing tick surveillance programs in India can help monitor tick populations and identify areas at risk for Lyme borreliosis transmission. By collecting data on tick distribution, researchers can better understand the factors driving the spread of Lyme borreliosis and develop targeted interventions to control tick populations. Collaborative research efforts between Indian and international institutions can also enhance our understanding of Lyme borreliosis ecology and contribute to the development of new diagnostic tools, vaccines, and treatments as the disease is more prevalent in regions like USA & Canada. Addressing the root causes of climate change is essential for reducing the long-term threat of Lyme borreliosis and other vector-borne diseases. Global efforts to mitigate climate change, such as reducing greenhouse gas emissions and transitioning to renewable energy sources, can help slow the rise in temperatures and limit the expansion of tick populations. At the national level, India can implement policies to promote sustainable land use, protect biodiversity, and conserve natural ecosystems. These efforts will not only help mitigate the impact of climate change but also reduce the risk of tick-borne diseases by preserving balanced ecosystems.

Conclusion

Lyme borreliosis is an emerging public health concern in India, driven in part by climate change and its impact on tick populations. While the disease remains relatively rare in the country, the increasing spread of tick infestation pan India and

the potential for underdiagnosis suggest that Lyme borreliosis may become a more significant issue in the coming years. Addressing the challenges of Lyme borreliosis in India requires a multi-faceted approach, including improving awareness among healthcare providers and the public, strengthening diagnostic and treatment protocols, and implementing preventive measures to reduce the risk of tick bites. Additionally, climate change mitigation efforts are essential for limiting the expansion of tick populations and reducing the long-term threat of Lyme borreliosis. By taking proactive steps to address the growing concern of Lyme borreliosis, India can protect its population from the potentially debilitating effects of this tick-borne illness and prevent its spread in the face of changing climate.

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Original Article

EXPLORING THE INFLUENCE OF METEOROLOGICAL FACTORS ON SCRUB TYPHUS INCIDENCE: AN ECOLOGICAL TIME SERIES ANALYSIS IN KERALA, INDIA

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ABSTRACT

Background: Scrub typhus, a re-emerging neglected tropical disease, now poses public health concerns in tropical and subtropical countries. Kerala, situated in the southwestern part of India, has been showing an increasing incidence of scrub typhus over the last few years. The dynamic nature of meteorological parameters such as temperature and rainfall influence the survival and activity of chiggers as well as the abundance and distribution of their rodent hosts. Consequently, elucidating these associations can

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aid in the development of early warning systems, targeted interventions and resource allocation to mitigate the impact of scrub typhus outbreaks in Kerala. In this study we aimed to analyse the relationship between meteorological factors (maximum temperature, minimum temperature and rain fall) and scrub typhus incidence in Kerala from 2012-2020.

Methods: Number of monthly reported scrub typhus cases for the period of 2012-2020 was obtained from Integrated Disease Surveillance Project (IDSP) of Directorate of Health Services, Kerala. Monthly district wise weather parameters viz. rain fall, maximum and minimum temperatures were obtained from India Meteorological Department (IMD), Pune. IMD daily gridded data was converted to district level. Influence of meteorological parameters on occurrence of scrub typhus was studied through correlogram using time series analysis technique.

Results: The present study shows that the maximum and the minimum temperatures negatively correlate with the scrub typhus incidence in Kerala. Rainfall has no influence on the scrub typhus incidence in Kerala. Temperature plays a more crucial role in predicting scrub typhus occurrence than seasonality or trends.

Conclusion: The study suggests a model of predicting the possibility of a scrub typhus incidence some three months in advance by observing the temperature conditions prevailing in a given area and also found that the climatic factor like rainfall do not show any significant influence on the occurrence of scrub typhus incidence in Kerala.

Keywords: Scrub typhus, temperature, rainfall, correlation

INTRODUCTION

Scrub typhus, a re-emerging neglected tropical disease, now poses public health concerns in tropical and subtropical countries. This disease is caused by *Orientia tsutsugamushi*, a bacterium belonging to the family Rickettsiaceae. The pathogen is transmitted from man to man by the bite of larvae of infected trombiculid mites (chiggers), which are ectoparasites of rodents and shrews[1]. The pathogen is transmitted from one stage to another through trans stadial and trans

ovarian transmission[2,3] The term "scrub typhus" originates from the presence of chiggers and rodents in scrub vegetation (shrub vegetation)[3].

Kerala, situated in the southwestern part of India, has been showing an increasing incidence of scrub typhus over the last few years[4]. The dynamic nature of meteorological parameters such as temperature and rainfall influence the survival and activity of chiggers as well as the abundance and distribution of their rodent hosts[5–7] Consequently, elucidating these associations can aid in the development of early warning systems, targeted interventions and resource allocation to mitigate the impact of scrub typhus outbreaks in Kerala.

In this study we aimed to analyse the relationship between meteorological factors (maximum temperature, minimum temperature and rain fall) and scrub typhus incidence in Kerala from 2012-2020.

Incidence of scrub typhus is highly influenced by meteorological factors like temperature, humidity and rainfall[8]. A study from China described the temperature and the scrub typhus association as initial elevated – descendent pattern. They found that heavy rainfall was associated with a sharp increase in scrub typhus risk[9]. In Japan, during 1984-2014, the average temperature in July and August of the previous year, cumulative rainfall in the September of previous year, snowfall throughout the winter and maximum depth of snow cover in January and February were positively correlated with number of scrub typhus cases[5]. Scrub typhus outbreak was reported in cooler months in Southern India[10].

Only a few studies have explored the influence of meteorological factors on the incidence of scrub typhus in India[7,10,11]. Given the variation in climatic factors from place to place and the re-emergence of scrub typhus in Kerala, we conducted a study on the influence of weather conditions on scrub typhus incidence in Kerala.

MATERIAL AND METHODS

The month wise data of scrub typhus cases in Kerala from 2012 – 2020 was obtained from the Integrated Disease Surveillance Project (IDSP) of Directorate of Health Services (DHS), Kerala[4]. The daily gridded rainfall, maximum and minimum temperature data from 2012–2020 was obtained from India Meteorological Department (IMD), Pune. The daily gridded weather data was

converted into district level data using Thiessen polygon approach using an area weighted average approach[12,13]. Further the district level data was compiled to get the state level data and the study design is ecological time series analysis.

STATISTICAL ANALYSIS

Influence of weather variables namely maximum temperature, minimum temperature and rainfall on the occurrence of scrub typhus in Kerala was studied through correlogram using time series analysis in R Statistical Software.[14] Trend analysis using decomposition of additive time series method was done from 2012 to 2020. Mann Kendal test was performed to check the trend of the occurrence of scrub typhus. Cross correlation plots of total monthly rainfall, monthly average maximum and minimum temperatures with number of reported cases were prepared.

RESULTS

In this study, we conducted a statistical analysis to explore the correlation between monthly scrub typhus cases and meteorological factors, including total rainfall, average maximum temperature, and average minimum temperature from 2012 to 2020. The results are summarized below**.**

Total number of scrub typhus reported in Kerala from 2012 to 2020 was 4064 (Table 1). Maximum number of cases were reported in December and January (Fig.1).

Table 1. Year-wise Incidence of Scrub Typhus in Kerala from 2012 to 2020

Year	No. of scrub typhus cases
2012	39
2013	68
2014	433
2015	1149
2016	633
2017	340
2018	400
2019	579
2020	423
Total	**4064**

Source: Directorate of Health Services, Kerala[4]

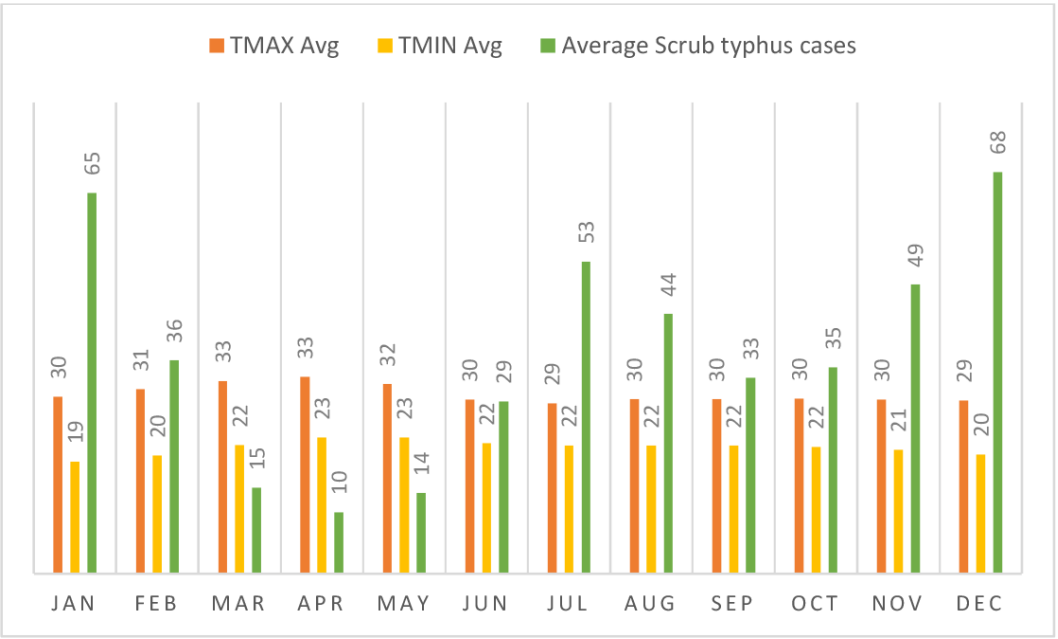

Fig. 1. Data on Maximum and Minimum Temperatures and scrub typhus incidence in Kerala from 2012 to 2020

CROSS-CORRELATION ANALYSIS OF AVERAGE MAXIMUM AND MINIMUM TEMPERATURES AND SCRUB TYPHUS INCIDENCE

Our analysis revealed a significant negative correlation between scrub typhus occurrence and the average maximum and minimum temperatures of the preceding three months. Specifically, an increase in both the average maximum and minimum temperatures during the previous three months was associated with a decrease in scrub typhus cases in the subsequent month. This relationship indicates a lag time of one month, meaning the effect of temperature changes manifests in scrub typhus incidence after a delay of approximately four weeks (Fig.1 and Fig.2).

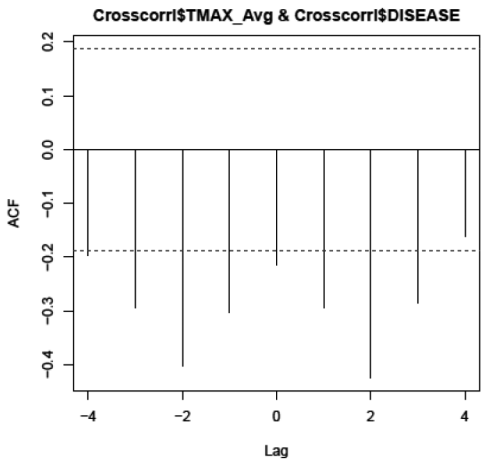

Fig. 2. Cross correlation plot of monthly average maximum temperature and average number of scrub typhus cases from 2012-2020

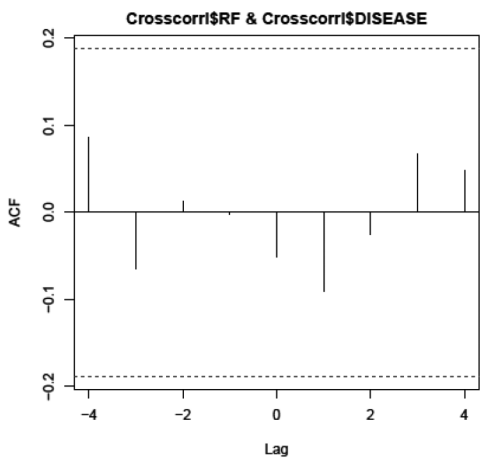

Fig. 3. Cross correlation plot of monthly total rainfall and average number of scrub typhus cases from 2012-2020

The analysis shows an evident trend and seasonality in scrub typhus occurrence in Kerala (Fig. 4). Further statistical testing using the Mann-Kendall test resulted in a tau value of 0.182, with a two-sided p-value of 0.0055516. This significant p-value indicates a statistically meaningful increasing trend in scrub typhus cases over time in the region (Fig.5)

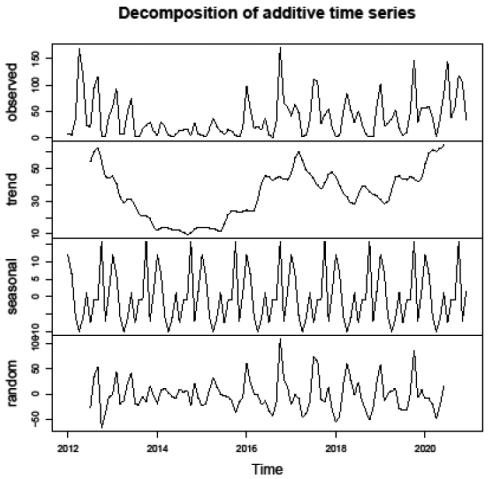

Fig. 4. Decomposition of Additive time series showing trend and seasonality of scrub typhus incidence in Kerala

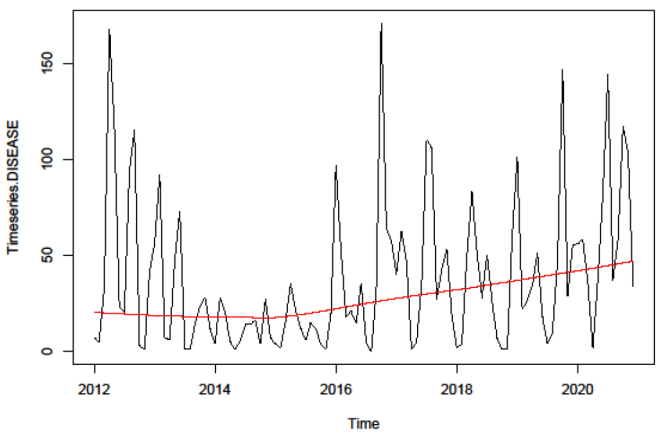

Fig. 5. Time Series Plot with trend showing increasing trend of scrub typhus incidence in Kerala

The results suggest that scrub typhus is becoming more prevalent in Kerala, with an upward trend. This trend should be closely monitored to assess the potential impact of climatic and environmental changes on disease transmission and to develop timely public health interventions.

Table 3. Impact of Relative Humidity on Disease Transmission across Regions

Region	Relative Humidity Range (%)	Scrub Typhus Incidence Rate (per 100,000)	Seasonal Trends	Reference
Global	60-90	5-20	Higher in monsoon season	CDC, WHO
Southeast Asia	70-85	15-30	Peaks in wet season	
India (Northeast)	75-95	25-40	Monsoon and post-monsoon	NCVBDC, India
India (North)	60-80	10-20	Monsoon and post-monsoon	Local epidemiology reports
China	65-85	8-15	Summer and monsoon	Local health reports, WHO
Japan	60-70	3-10	Early summer	Local epidemiology reports
Australia	50-70	2-5	Summer	WHO, Local government health reports
Indian Subcontinent	65-90	20-35	Monsoon	CDC, WHO

The table 3 indicate a strong correlation between higher relative humidity, typically above 60%, and increased scrub typhus incidence. Regions like Southeast Asia and Northeast India, where humidity levels often range between 75-95%, report incidence rates reaching 30-40 cases per 100,000 individuals, suggesting that high humidity may create favorable conditions for the vectors and hosts of *Orientia tsutsugamushi*, the bacterium responsible for scrub typhus. Seasonal patterns also influence incidence rates, with regions experiencing wet or monsoon seasons, such as India, Southeast Asia, and parts of China, seeing more cases during these periods. In India's Northeast and North regions, cases surge during the monsoon and post-monsoon months, likely due to the lifecycle of the mites that transmit scrub typhus, which thrive in warm, humid environments. Globally, there is significant variation in incidence rates, with Southeast Asia and parts of India exhibiting the highest rates, while Japan and Australia report lower rates, potentially due to differences in vector distribution, environmental conditions, and

public health measures. Notably, Japan's incidence peaks in early summer when humidity is moderate, underscoring regional variability in seasonal patterns. These findings highlight the importance of factoring in environmental aspects such as humidity and seasonal climate for public health strategies. Areas with high humidity and predictable monsoon seasons could benefit from targeted interventions, including awareness campaigns, surveillance, and preventive actions during peak transmission times, to help mitigate the spread of scrub typhus.

Table 4: Impact of Temperature on Disease Transmission across Regions

Region	**Optimal Temperature Range (°C)**	**Disease Incidence Rate (per 100,000)**	**Transmission Pattern**	**Source**
Tropical Regions	25-30	20-50	High year-round due to consistent warmth	WHO CDC Local health agencies
Southeast Asia	24-28	30-60	Peaks in summer and monsoon	
India (Northern)	20-35	10-40	Increases in summer and monsoon	National Centre for Vector Borne Diseases Control (NCVBDC)
Sub-Saharan Africa	22-32	15-45	High during warmer months	Local epidemiology reports
South America	23-29	10-30	Peaks during warmer, wetter months	WHO, Local health agencies
Temperate Regions	15-25	5-15	Peaks in summer	Local health agencies

The table 4 illustrates the impact of temperature on disease transmission across various global regions, highlighting optimal temperature ranges for vector activity and corresponding disease incidence rates. In tropical regions, where temperatures consistently range between 25-30°C, disease incidence remains high year-round, with rates from 20 to 50 cases per 100,000 as the warmth supports continuous vector breeding. Southeast Asia and Northern India show similar patterns, where temperatures between 24-35°C during summer and monsoon seasons result in heightened disease rates, peaking between 30-60 and 10-40 cases per 100,000 respectively. Sub-Saharan Africa, with an optimal range of 22-32°C, experiences higher transmission during warmer months, with incidence rates from 15 to 45 per

100,000 as noted in local reports. South America sees a spike in cases, 10-30 per 100,000 during warmer, wetter months, aligning with its 23-29°C range. In temperate regions, where ideal temperatures fall between 15-25°C, disease incidence peaks in summer but remains lower, between 5 and 15 per 100,000.

DISCUSSION

Scrub typhus is an emerging vector borne disease in Kerala[4]. Its incidence is influenced by many factors like vector population[15] and meteorological factors[9].

The present study provides valuable insights into how climatic factors impact the incidence of scrub typhus in Kerala. The findings have significant implications for public health planning and disease prevention in the region. In this study we assessed the impact of temperature and rain fall on the occurrence of scrub typhus in Kerala using data from 2012 to 2020 of the State. The study shows that the number of scrub typhus cases are showing increasing trend and seasonality in Kerala.

In our study average maximum temperature and average minimum temperature had a negative correlation with number of scrub typhus cases. The same trend was found in Vellore, Tamil Nadu[7]. A study from China shows scrub typhus incidence was positively correlated when mean temperature was at 19-20^{o}C and in Japan, scrub typhus cases increased when average temperatures were at 22.5^{o}C and 24.1^{o}C.[5,16] The present study also shows that the maximum number of cases occurred when average maximum and minimum temperatures were at 29^{0}C and 20^{0}C respectively. The average minimum temperature at which maximum number of cases occurred in the present study is in agreement with the above reported studies. A multi-centric study in Taiwan reported a positive correlation between scrub typhus cases and temperature, with the highest number of cases occurring when the temperature ranged between 25°C and 30°C[17]. Similarly, in China, the association between temperature and scrub typhus followed an initial elevated–descendant pattern, with the highest number of cases occurring at 24.5°C [9]. The favourable temperature for scrub typhus occurrence in the present study is in conformity with the findings from the above referred studies conducted in various geographical locations. One reason for the impact of temperature on scrub typhus incidence is its influence on chigger activity. The favourable temperature for egg laying of mites was between 23^{o}C and 25^{o}C. There was no egg laying when

temperature was less than 20^{o}C and more than 30 ^{o}C[18]. Frances *et al*. reported that scrub typhus infection increases when chigger population is high and more chiggers were attached to *Rattus rattus* during wetter months of the year[19]. This suggests that temperature plays a more crucial role in predicting scrub typhus occurrence than seasonality or trends.

Many studies have reported a positive correlation between rainfall and scrub typhus occurrence[7,9,16]. In Japan, cumulative rainfall in September of the previous year showed a positive correlation with scrub typhus incidence. But cumulative rainfall in July of the previous year showed a negative correlation with disease incidence. According to authors, too much rainfall in July might interfere with fertilization by washout of male mites or spermatophores around the females[5]. Studies from China reported that there was a sharp increase in scrub typhus incidence with a heavy rainfall[9,16]. The present study found no relationship between scrub typhus incidence and monthly average rainfall in Kerala. This could be due to the fact that averaging rainfall across all districts could potentially mask localized relationships. Rainfall can indeed vary significantly across Kerala due to its diverse topography, ranging from coastal to mountainous regions.

The results suggest that scrub typhus is becoming more prevalent in Kerala, with an upward trend. This trend should be closely monitored to assess the potential impact of climatic and environmental changes on disease transmission and to develop timely public health interventions. The data underscores the role of temperature in influencing vector-borne disease transmission, with warmer climates and seasons creating higher risk periods and highlighting the need for climate-sensitive public health responses.

Climatic and environmental changes play a crucial role in disease transmission by creating favorable conditions for vectors and pathogens. Warmer temperatures, increased humidity, and heavy rainfall can accelerate vector breeding and prolong pathogen survival, leading to higher incidences of diseases like malaria, dengue, and scrub typhus. Urbanization and deforestation further increase human exposure to wildlife reservoirs and vectors. Timely, adaptive public health interventions—such as vector surveillance, early-warning systems, and community-level measures like insecticide-treated nets and improved sanitation—are essential to mitigate these risks. Aligning public health strategies with environmental changes enables more effective disease prevention and protection for vulnerable populations.

While most studies have reported a positive correlation between rainfall and scrub typhus occurrence, our study did not find any significant association[7,9,16]. This discrepancy may be influenced by region-specific environmental factors, human outdoor activity and vector biology. In the future, incorporating vector biology alongside meteorological factors will be crucial for improving scrub typhus prediction models.

We could not analyse the correlation between district wise disease incidence and meteorological parameters. Bionomics of the vector also plays an important role in disease re-emergence. However, we observe that the incidence of scrub typhus occurrence is showing an increasing trend in the last few years in Kerala and the future research on the influence of weather parameters on vector bionomics could give more clear information on this increasing trend of scrub typhus incidence in Kerala.

CONCLUSION

The present study demonstrates an increasing trend in scrub typhus cases in Kerala over the period of study. Both maximum and minimum temperatures were found to be negatively correlated with scrub typhus occurrence. Our findings suggest that temperatures three months prior to disease incidence significantly influence the likelihood of scrub typhus outbreaks. Therefore, it may be possible to predict scrub typhus outbreaks up to three months in advance. However, no significant relationship was observed between cumulative rainfall and the occurrence of scrub typhus in Kerala. Understanding the influence of weather factors on disease dynamics can help to develop predictive models for forecasting outbreaks.

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Original Article

EXTRACTS FROM KINNOW PEEL WASTE AS LARVICIDAL AGENTS AGAINST DENGUE SPREADING VECTOR, *AEDES AEGYPTI*

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ABSTRACT

Background: Mosquitoes, in particular, *Aedes aegypti* pose a serious threat to human health as they act as vectors for the transmission of several deadly diseases namely dengue, chikungunya, yellow fever and Zika. The present research is targeted towards the evaluation of larvicidal property of extracts prepared from kinnow peel waste against *Ae. aegypti*.

Methods: Ethanolic and aqueous extracts were prepared from kinnow peel waste followed by agitation for 24 hours in an orbital shaker. Larvae of *Ae. aegypti* (L4) were exposed to laboratory prepared five different concentrations of kinnow peel

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ethanolic extract @ 0.05, 1.0, 1.5, 2.0 and 2.5% and aqueous extract @ 6.0, 7.0, 8.0, 9.0 and 10.0%. Effective larvicidal concentration of each of the kinnow peel extracts along with their storage and retention efficacy was also determined.

Results: Out of the tested concentrations, 2.0% ethanolic extract and 9.0% of aqueous extract from kinnow peel waste were found to be the effective larvicidal concentrations against *Ae. aegypti*. After 12 hours of exposure, LC_{50} and LC_{90} toxicity values were calculated to be 0.07 and 0.16% for ethanolic and 6.41 and 8.23% for aqueous extracts, respectively. Larvicidal retention efficacy of the effective concentration of these extracts persisted up to 12 hours. A significant delay in development (from L4 to adult) was also observed after placing new larvae in the left over effective concentration of these extracts. No effect of storage (for 6 months) on the larvicidal potential of kinnow peel extracts was observed.

Conclusion: Present study showed the larvicidal property of extracts prepared from kinnow peel waste against *Ae. aegypti* revealing their potential to be used as eco-friendly mosquito control agents in future.

Keywords: *Aedes aegypti*; development duration; kinnow peel waste; larvicidal potential; mosquito control; retention efficacy

INTRODUCTION

Aedes mosquito serves as vector for some of the world's threatening diseases like dengue, chikungunya, yellow fever and zika fever[1]. The two primary vectors responsible for the proliferation of viral disease dengue are *Aedes aegypti* and *Ae. albopictus* and among both, *Ae. aegypti* has higher rate of transmitting this disease in India[2]. *Ae. aegypti* is abundantly present in small freshwater collections like roadside ditches, earthen pots, desert coolers, gardens, small containers etc, lying in peri-domestic areas[3]. Last year, 2,89,235 dengue cases have been recorded in India by National Vector Borne Disease Control Programme[4]. The control and prevention of dengue disease entirely depends on the effectiveness of strategies and measures being implemented towards the control of this dreadful vector. Use of insecticides is a common practice in mosquito control and many synthetic/chemical insecticides are being widely used for controlling adult and larval mosquito population at their breeding locations. Synthetic insecticides used for mosquito control include permethrin, allethrin and malathion along with some insect repellents like DEET

(N, N-Diethyl-meta-toluamide) and picaridin[5]. However, studies have shown that these compounds when used intensively results in affecting humans, the non-target organisms, and the environment, due to their residual effects along with increasing resistance among mosquito population[6]. Because of their potentially virulent effects, high operational costs and environmental contamination, there is an urgent need for the development and implementation of effective, economic and eco-friendly alternative ways to control mosquitoes and associated diseases.

The plant based products are found to have insecticidal properties which has resulted in significant interest of researchers in botanicals as potential sources of natural mosquito control material in recent years. Nearly 86 compounds extracted from plants have been observed to have larvicidal potential against *Ae. Aegypti*[7,8]. Essential oil (EOs) and extracts of plant origin are the mixtures of major as well as minor constituents, which act synergistically in the target individuals in contrast to synthetic insecticides. Moreover, these plant based products are environmentally safe and biodegradable[9] and also have been found to be potent against those species of insects which are resistant to synthetic products[10]. Citrus is an important fruit crop that is mostly grown in tropical or subtropical climates[11]. Kinnow, a high yielding mandarin hybrid variety (a cross between *Citrus nobilis* and *C. deliciosa*) grown in Punjab, has become well-known among citrus fruits. The peel of this fruit, which accounts for around 30-34% of the total weight[12] is typically considered as trash, although it is more valuable due to the presence of numerous active phytochemicals with potential larvicidal characteristics[13]. By utilizing kinnow peel, the plant wastes can be reduced and assessed for further usage in a beneficial manner. Keeping this in mind, the current study was planned to evaluate the larvicidal potential of ethanolic and aqueous extracts derived from kinnow peel waste against *Ae. aegypti*.

MATERIAL AND METHODS

***Collection of Ae. aegypti larvae*:** Water samples were taken from various small fresh water collections such as desert coolers, roadside ditches, plates under pots, rubber tyres, plastic containers and earthen pots located in peri-domestic areas of urban regions in the Ludhiana district of Punjab state (India). *Ae. aegypti* larvae were identified and separated from other types of mosquito larvae (if present) from these collected water samples using standard keys based on their morphological characters[1].

***Collection of kinnow peels*:** Fresh kinnow peels were collected from the various local fruit vendors of Ludhiana city. The collected peels were cleaned and separated from the pulp, leaves and stem of the fruit. Peels that were infected were discarded.

***Preparation of kinnow peel extracts*:** The collected fresh kinnow peels were shade dried on filter paper at room temperature for 5-6 days till they got brittle and completely dried. Dried peels were finely pulverized using a grinder to make fine powder. For preparing kinnow peel extracts, 20 g of kinnow peel powder was taken in conical flask, followed by addition of 100 ml ethanol for ethanolic extract and 100 ml distilled water for the preparation of aqueous extract. The flasks were covered with aluminum foil and then kept in an orbital shaker for frequent agitation at 25° C temperature at 80 rpm for mixing over a period of 24 hours. Then the mixture was filtered using muslin cloth followed by filter paper and filtrate/extracts so obtained were used for further testing.

***Dose response larvicidal bioassay of kinnow peel extracts against Ae. aegypti*:** To determine the larvicidal potential, preliminary testing of freshly prepared kinnow peel ethanolic and aqueous extracts was carried out against *Ae. aegypti* larvae (L4) by random selection of higher and lower concentrations of these extracts. On the basis of preliminary screening, five different concentrations of kinnow peel ethanolic extract @ 0.05, 1.0, 1.5, 2.0 and 2.5% and aqueous extract @ 6.0, 7.0, 8.0, 9.0 and 10.0% were prepared by mixing the required volume of each of kinnow peel extract in 1 ml of dimethyl sulphoxide (DMSO, a non-polar and non-toxic emulsifying agent) and total volume was made up to 250 ml with de-chlorinated water (prepared by keeping the tap water in opened buckets for overnight). *Ae. aegypti* L4 larvae (n=20) were exposed to five different concentrations of the prepared kinnow peel ethanolic and aqueous extracts in plastic beakers of 250 ml capacity covered with muslin cloth using a rubber band. A control set (having 250 ml of de-chlorinated water) and a vehicle-control set (having 1 ml DMSO and 249 ml of de-chlorinated water) were also run simultaneously. All experimental sets were run in triplicate and larvae were fed adequately with dog biscuits and yeast ground in ratio 3:1 (added @ 2mg/100ml) till their transformation to next non-feeding pupal stage. Larval mortality was recorded after 3, 6, 9, 12, 24, 36 and 48 hours of treatment in ethanolic and aqueous extract treated, control and vehicle-control sets. The larvae were considered dead, if they were unable to respond or move when stimulated using a brush. The dead larvae in each set were counted and

removed from the experimental sets. Out of the tested concentrations, the minimum concentration of ethanolic and aqueous extracts showing maximum mortality was considered as the effective concentration, which was used for further experimentation purpose. For calculating LC_{50} and LC_{90} after 12 hours of post-exposure, the log concentration-mortality regression was worked out by log probit technique[14] employing the computer programme POLO[15].

***Retention activity period of kinnow peel extracts*:** For assessing the larvicidal retention activity period of kinnow peel extracts, fresh *Ae. aegypti* L4 larvae (n=20) were kept in the leftover tested solution of effective concentration of kinnow peel ethanolic and aqueous extracts (assessed during larvicidal bioassay of respective extracts) after removing all the dead larvae from the beakers. After 3, 6, 9, 12, 24, 36 and 48 hours, dead larvae (if any) were counted and replenished with the same number of the new larvae so as to have total number of larvae as 20 (which were taken initially). Control and vehicle-control sets were also kept simultaneously along with treatment trials in triplicate. In another experiment, fresh *Ae. aegypti* L4 larvae (n=20) were introduced in properly covered beakers containing the leftover tested solution of the effective concentration of kinnow peel ethanolic and aqueous extracts and were further monitored to determine the retention activity effect of these extracts (after removing all the dead larvae of the larvicidal bioassays) on larval development duration i.e. time taken by L4 larvae to pupae and from pupae till adult emergence. The muslin cloth was removed from the beakers when the larvae got transformed into pupae and then these beakers were kept in mosquito rearing cages. Emerged adults were fed on sugary juice of deseeded water-soaked raisins kept in a sterilized petri plate already placed inside mosquito rearing cages. To provide them water, a moist cotton swab was also placed on the top of each cage. After the completion of experiments, emerged mosquitoes were killed by keeping chloroform dipped cotton swabs inside the mosquito rearing cages. Control and vehicle-control sets were also kept simultaneously along with treatment sets in triplicate. The time taken for each transformation (i.e. from L4 to pupa and pupa to adult) was recorded along with recording of per cent adult emergence in all the sets.

***Larvicidal potential of stored kinnow peel extracts*:** Kinnow peel ethanolic extract was kept in clean glass vials covered with aluminum foil and placed at 4º C in the refrigerator, while aqueous extract was kept in properly covered plastic vials at -18º C in deep freezer and both the extracts were stored for 2, 4 and 6 months. The

already determined effective larvicidal concentrations (tested during larvicidal bioassay) were tested again for the freshly prepared and stored (2, 4 and 6 months old) ethanolic and aqueous extracts against *A*e. *aegypti* by following the same procedure and larval mortality was recorded at regular intervals of three hours in all the sets.

***Statistical analysis*:** The data was statistically analyzed by comparing the larval mortality and developmental duration recorded in kinnow peel ethanolic and aqueous extract treated sets with that of control and vehicle-control sets in their respective experimental trials and larvicidal potential of stored kinnow peel ethanolic and aqueous extract treated sets with that of their respective freshly prepared extracts by one way analysis of variance (Duncan multiple range test).

RESULTS

Dose response larvicidal bioassay of kinnow peel extracts against Ae. aegypti

When *Ae. aegypti* larvae (L4) were exposed to 0.05% of kinnow peel ethanolic extract, 13.33±2.88% mortality was observed within 3 hours, which was observed to increase up to 26.67±5.77% till 12 hours and after that, no further larval mortality was found. A similar trend in larval mortality was observed after exposing the larvae to 0.10 and 0.15% concentrations of ethanolic extract. However, exposure of larvae to 0.20 and 0.25% of ethanolic extracts resulted in 100%mortality within 6 and 3 hours respectively. Per cent larval mortality was found to increase statistically with increase in the concentration of ethanolic extract at the respective hours of each treatment. However, no larval mortality was observed in control and vehicle-control set (Table 1). Exposure of L4 *Ae. aegypti* larvae to 6.0% kinnow peel aqueous extract resulted in 28.33±7.63% mortality which increased up to 41.67±5.77% till 12 hours and there after that no further larval killing was reported. Increase in larval mortality was recorded with increase in concentration of aqueous extract and at 9.0 and 10.0% concentrations, 100% larval killing was recorded respectively within 12 and 9 hours of exposure (Table 2). Various toxicity values i.e. LC_{50} and LC_{90} of kinnow peel extracts computed for *Ae. aegypti* larvae based on record of their mortality for the exposure of 12 hours were worked out to be 0.07 and 0.16% for ethanolic extract and 6.41 and 8.23% for aqueous extract, respectively (Tables 1 and 2). Kinnow peel ethanolic extract @ 0.20% and aqueous extract @ 9.0% were found to be the effective larvicidal

concentratons, as these resulted in maximum larval mortality (100%) with minimum concentration in comparison to that of all the respective tested concentrations (Fig.1).

Retention activity period of kinnow peel extracts

Freshly added *Ae. aegypti* L4 larvae exposed to the effective concentration of ethanolic (0.20%) and aqueous (9.0%) extract (determined during larvicidal bioassay in Tables 1 and 2) showed no larval killing after 3, 6, 9, 12, 24, 36 and 48 hours in any of the triplicate sets. However, a significant delay in duration of time taken from L4 to adult emergence was recorded during the development of these larvae exposed to each of kinnow peel extract. In control set the average time taken for the transformation of L4 larval stage to pupa and from pupa to adult was recorded to be 2.66±1.15 and 1.16±0.28 days and in vehicle-control set it was found to be 2.5±0.86 and 1.33±0.28 days. Retention efficacy effect in terms of delayed development that is exposure of leftover effective concentration (0.20%) of kinnow peel ethanolic extract resulted in significant delay in development of L4 to pupa and pupa to adult, as it took 5.00±0.00 and 1.83±0.28 days respectively. Similarly, exposure of L4 stages of *Ae. aegypti* to leftover effective concentration of kinnow peel aqueous extract (9.0%) resulted in significant delay in development of L4 to pupa and pupa to adult i.e. 4.83±0.28 and 2.00±0.00 days respectively. Overall development from L4 stage till adult formation in ethanolic and aqueous extract treated sets was observed to take statistically longer duration almost double time i.e. 6.83±0.28 and 6.50±0.50 days respectively as compared to control (3.82±1.43 days) and vehicle-control sets (3.83±1.14 days). However, there was no effect of treatment of kinnow peel extracts on the adult emergence, as it was found to be 100% in all the sets (Table 3).

Table 1. Mortality of *Aedes aegypti* larvae being exposed to different concentrations of kinnow peel ethanolic extract

Concentration (%)	Per cent mortality up to (Mean±S.D)							Maximum mortality (%)
	3hr	6hr	9hr	12hr	24hr	36hr	48hr	
0.05	13.33±2.88[a]	16.67±5.77[a]	21.67±2.88[a]	26.67±5.77[a]	NFM	NFM	NFM	26.67±5.77[a]
0.10	31.67±5.77[b]	51.67±7.63[b]	53.33±5.77[b]	63.33±2.88[b]	NFM	NFM	NFM	63.33±2.88[b]
0.15	58.33±5.77[c]	61.67±2.88[b]	66.67±2.88[c]	76.67±7.63[c]	NFM	NFM	NFM	76.67±7.63[c]
0.20	76.67±2.88[d]	100.00±0.00[c]	-	-	-	-	-	100.00±0.00[c]
0.25	100.00±0.00[e]	-	-	-	-	-	-	100.00±0.00[e]
0 (Control)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0 (Vehicle-control)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Toxicity Value (%)	Fiducial limits		χ^2
	Lower limit	Upper limit	
LC_{50}= 0.07	0.3	0.11	11.25
LC_{90}= 0.16	0.11	0.45	

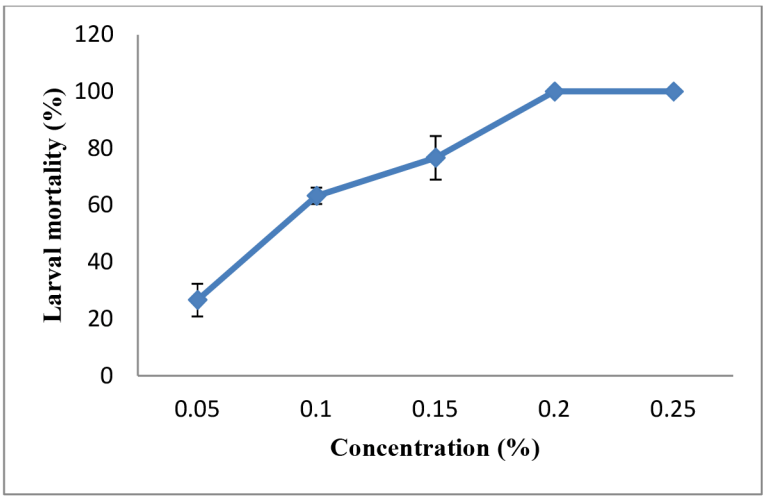
- NFM represents no further mortality
- Figures followed with different superscripts indicate significant difference ($p<0.05$) with respect to different treatments by using Duncan multiple range test

Table 2. Mortality of *Aedes aegypti* larvae being exposed to different concentrations of kinnow peel aqueous extract

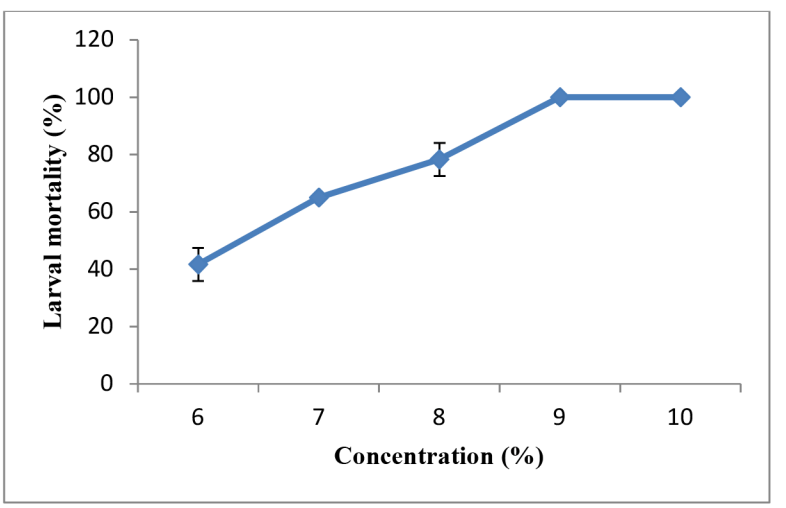
Concentration (%)	Per cent mortality up to (Mean±S.D)							Maximum mortality (%)
	3hr	6hr	9hr	12hr	24hr	36hr	48hr	
6.0	28.33±7.63[a]	33.33±7.63[a]	38.33±2.88[a]	41.67±5.77[a]	NFM	NFM	NFM	41.67±5.77[a]
7.0	31.67±7.63[a]	36.67±2.88[a]	43.33±5.77[a]	65.00±0.00[b]	NFM	NFM	NFM	65.00±0.00[b]
8.0	40.00±5.00[b]	46.67±2.88[b]	53.33±2.88[b]	78.33±5.77[c]	NFM	NFM	NFM	78.33±5.77[c]
9.0	51.67±2.88[c]	58.33±7.63[c]	83.33±7.63[c]	100.00±0.00[d]	-	-	-	100.00±0.00[d]
10.0	66.67±2.88[d]	81.67±5.77[d]	100.00±0.00[d]	-	-	-	-	100.00±0.00[d]
0 (Control)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0 (Vehicle-control)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Toxicity Value (%)	Fiducial limits		χ^2
	Lower limit	Upper limit	
LC_{50}= 6.41	4.99	7.06	8.18
LC_{90}= 8.23	7.44	11.31	

- NFM represents no further mortality
- Figures followed with different superscripts indicate significant difference ($p<0.05$) with respect to different treatments by using Duncan multiple range test



(A)



(B)

Fig. 1. Maximum mortality (%) of *Aedes aegypti* larvae after exposing to different concentrations of kinnow peel extracts. (A) Ethanolic extract and (B) Aqueous extract

Table 3. Retention activity of effective concentration of kinnow peel extracts in terms of development and emergence of *Aedes aegypti*

Experimental set	Duration of developmental period in days (Mean±S.D)			Per cent adult emergence (Mean±S.D)
	L4-Pupa	Pupa-Adult	L4-Adult	
Control	2.66±1.15a	1.16±0.28a	3.82±1.43a	100.00±0.00a
Vehicle-control	2.5±0.86a	1.33±0.28a	3.83±1.14a	100.00±0.00a
Ethanolic extract (0.20%)	5.00±0.00b	1.83±0.28b	6.83±0.28b	100.00±0.00a
Aqueous extract (9.0%)	4.83±0.28b	2.00±0.00b	6.50±0.50b	100.00±0.00a

- Figures followed with different superscripts indicate significant difference ($p<0.05$) with respect to Control and Vehicle-control sets by using Duncan multiple range test

Larvicidal potential of stored kinnow peel extracts

When *Ae. aegypti* L4 larvae were exposed to effective concentration i.e. 0.20% of fresh and stored (2, 4 and 6 months) ethanolic kinnow peel extract, statistically similar per cent larval mortality after 3 hours and 100% mortality after 6 hours was observed. Similarly, effective concentration of aqueous kinnow peel extract i.e. 9.0% of fresh and those stored for 2, 4 and 6 months, resulted in statistically similar per cent mortality up to 9 hours and 100% killing of *Ae. aegypti* larvae after 12 hours in all cases. Results clearly indicated no effect of storage on larvicidal activity of both the types of prepared kinnow peel extracts.

DISCUSSION

Citrus species are well known for their economic importance and thus are widely cultivated fruits globally. Citrus plant contains several important phytochemicals[16] out of these limonoids exhibit a wide range of biological activities including insect repellent and larvicidal[17]. Kaur *et al.*[18] have observed limonene as the most abundant compound (64.82%) in kinnow peel oil which is mainly considered to be responsible for its larvicidal action by acute toxicity against *Ae. aegypti*,being mainly involved in arresting the metabolic activities of larvae. In the

same study, the other major constituents of the kinnow peel oil reported were elemol (8.28%), β-citronellal (4.34%), geraniol (3.58%), viridifloral (2%), N, N-dimethylacetamide (DMA) (1.91%), β-elemene (1.64%), β-citronellol (1.52%), β-myrcene (1.34%) and germacra-1,4,5-triene (1.07%). Ethanolic extract from the pulp of *C. aurantifolia* when tested against *Ae. albopictus*, was found to possess larvicidal property[19]. In another finding *Cambly carpa* ethanolic extract exhibited promising larvicidal effects against *Ae. aegypti*[20]. The larvicidal activity of prepared kinnow peel extracts observed during the present study is due to the presence of such secondary metabolites. During the present study, it was observed that both the type of extracts (ethanolic and aqueous kinnow peel extracts) showed no larval killing after 12 hours of exposure, indicating highly volatile characteristics of these extracts. Actually kinnow peel has high content of limonene, which is unstable in light and is highly volatile in nature[18]. Due to this reason very low retention efficacy of the prepared extracts was observed. Volatility of plant based products (oils/extracts) vary from plant to plant species, as certain phytochemicals are stable for few hours and others may show their stability up to several days. However, rapid action of these phytochemicals cause acute neurotoxicity, resulting in knockdown effect on the larvicidal populations[21] as also seen during the present research.

Plant-based products exhibit growth inhibiting effects on the various developmental stages of different mosquito species due to a variety of pre-emergent defects such as prolongation of instars and pupal duration, inhibition of larval and pupal moulting, morphological abnormalities and mortality due to toxicity during the developmental phases[12]. Inhibition/delay in development of *Ae. aegypti* larvae along with morphological and histological alterations have been observed after their exposure to curcumin/d-mannitol[22]. Similarly, the current study also found a significant delay in duration of time taken from L4 to adult emergence during the developmental period of the larvae after exposure to kinnow peel extracts. Though the larvicidal retention activity of kinnow peel extracts was found to be only up to 12 hours, but after that freshly added L4 instars of *Ae. aegypti* in the leftover extract solutions of treatment trials showed significant delay in overall development (L4 to adult) indicating retention activity of these extracts in terms of increasing the development duration. Delayed development have also been recorded in *Ae. aegypti* larvae after treatment with extracts from seeds of *Adenanthera pavonina*, which possibly may be because of defective proteolysis in the larvae's midgut[22]. Extract

from *Brucea* spp leaves[24]and *Eucalyptus* oil nanoemulsion[13]have been observed to cause developmental delay in *Ae. aegypti* larvae. Actually, moulting and metamorphosis in insects depends on several growth hormones and disruption of growth hormone production may cause inhibition and delay in larval growth and development to pupa and then to adult[25].

Oils/extracts of plant origin get easily degraded, when stored at room temperature and to a lesser extent when kept in the dark. Thus, if these extracts are properly stored to prevent oxidation and polymerization caused by air and light, they remained stable and effective too. During the present study, it was observed that the larvicidal potential of kinnow peel ethanolic and aqueous extracts was found to remain unaffected even kept for 6 months because of their proper storage in cold and dark conditions in closed vials which effectively prevented autoxidation. Santos *et al*.[26] have observed that larvicidal properties of EOs against *Ae. aegypti* remained unaffected even up to three years of storage kept under proper conditions.

CONCLUSION

The present study concluded that kinnow peel ethanolic extract @ 0.20% and aqueous extract @ 9.0% were found to have efficient larvicidal potential against *Ae. aegypti* L4 instars. These extracts showed retention activity in terms of significantly delaying the development duration (L4 to adult). No effect of storage (even up to six months) on the larvicidal activity of kinnow peel ethanolic and aqueous extracts was observed. This study highlighted the significance of kinnow peel (generally treated as waste byproduct) as an important source of different bioactive compounds exhibiting larvicidal properties. However, the issue of high volatility of kinnow peel extracts necessitates the addition of a stabiliser or fixative to enhance the sustainability and stability of these extracts leading to the preparation of any formulation in future. Thus, such extracts can be exploited for managing the problem of dengue transmitting vector, *Ae. aegypti*.

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AUTHOR CONTRIBUTION STATEMENT

DK conceived and designed research problem. AK conducted experiments. AK and DK analyzed data. DK and AK prepared, edited and finalized the manuscript.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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Original Article

CROSS-RESISTANCE TO DIFFERENT CLASSES OF INSECTICIDES IN PYRETHROIDS-SELECTED *CULEX QUINQUEFASCIATUS*

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ABSTRACT

Background: *Culex quinquefasciatus* is the vector of several life-threatening diseases. In addition to spreading disease, it is a source of irritation due to its relative abundance. Vector control strategies particularly chemical control are employed to combat the spread of vector-borne diseases. Synthetic pyrethroids such as permethrin (Type I) and alphacypermethrin (Type II) are recommended for indoor residual spray, long-lasting insecticidal treated nets, outdoor fogging and liquid vaporizer mosquito repellent.

Methods: In this study, three strains of mosquitoes namely alphacypermethrin-selected (AS), permethrin-selected (PS), and susceptible (S) strains were reared for

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20 generations under selection pressure of alphacypermethrin, permethrin and in the absence of insecticide respectively. The susceptibility status of these three strains against different classes of insecticides with and without the use of synergists was analysed. Synergists were used to examine the involvement of metabolic resistance mechanisms. Biochemical assays and allele-specific polymerase chain reactions were conducted to study the resistance mechanism involved.

Results: It was found that both the selected strains showed high resistance and cross-resistance to pyrethroids and other classes of insecticides as the mortality percentage was less than 90% except in the case of malathion. Even the use of synergists could only partially restore the susceptibility. Biochemical assays showed higher activities of monooxygenases and fixation of L1014F *kdr* mutation in the selected strains.

Conclusion: Both metabolic detoxification and *kdr* mutation were involved in the resistance in pyrethroid-selected strains, resulting in cross-resistance. Hence, monitoring the insecticide susceptibility status before the application of a particular insecticide is extremely essential to know its efficiency in controlling the vector. Moreover, rotation of insecticides with different modes of action is highly encouraged to increase the effectiveness of vector control.

Keywords: Alphacypermethrin, permethrin, WHO Tube test, Synergist test, Biochemical assays, kdr genotyping

INTRODUCTION

Culex quinquefasciatus is the vector of several life-threatening diseases such as lymphatic filariasis (LF), Japanese encephalitis (JE), West Nile virus (WNV), Saint Louis encephalitis virus (SLEV), Usutu virus, avian malaria, dog heartworm, Rift valley fever, etc. In addition to spreading diseases, *Culex* mosquitoes are a source of nuisance. *Culex quinquefasciatus* is the most abundant mosquito, found in tropical and subtropical regions[1]. In India, it is the predominant vector of the nematode worm *Wuchereria bancrofti*, that causes bancroftian filariasis[2]. LF is endemic to 345 districts from 21 States and Union Territories (NCVBDC, accessed 20 October 2024). Mass drug administration (MDA) of a combination of three drugs namely ivermectin, diethylcarbamazine citrate (DEC) and albendazole in regular intervals has been the primary control method of LF[3]. However, poor

community participation and low compliance with MDA have created a barrier to its complete eradication in India. In this situation, vector control aids in combating the spread of diseases.

Vector control mainly depends upon the chemical control method, especially during epidemic conditions for the quick reduction of vector population and subsequent control over the disease. Organochlorines, organophosphates, carbamates, synthetic pyrethroids, neonicotinoids, phenylpyrazoles, butenolides, diamides and insect growth regulators are different classes of chemical insecticides commonly used for vector control. Synthetic pyrethroids, used as indoor residual spray, long-lasting insecticidal treated nets, outdoor fogging, and liquid vaporizer mosquito repellent have dominated the field of chemical control due to their fast action and low mammalian toxicity[4]. Pyrethroids disrupt the functioning of the voltage-gated sodium channel in both insects and mammals[5]. They are divided into type I and type II depending upon the structure (absence and presence of α cyano group respectively), symptoms of poisoning (hyperexcitation, prostration, body tremors, and stimulus-dependent nerve depolarization) and electrophysiological responses (repetitive discharge of action potential and disruption of action potential)[6]. Some pyrethroids show intermediate functions and are difficult to distinguish into types[7]. Alphacypermethrin is a type II synthetic pyrethroid used as an agricultural pesticide[8] as well as for vector control[9]. Permethrin is a type I synthetic pyrethroid used against various insect pests and vectors[10].

However, in response to the insecticidal selective pressure, mosquitoes are parallelly developing resistance against these insecticides making them less effective. Insecticide resistance has been reported profoundly in the *Culex quinquefasciatus* population across the globe as they are continuously exposed to the insecticides directly and indirectly[6,11–13]. Resistance to insecticides is achieved through different mechanisms individually or in combination. Target-site mutation and metabolic resistance are the major resistance pathways for pyrethroid resistance[14]. Resistance against pyrethroids is mainly conferred by the knockdown resistance (kdr) mutation in voltage-gated sodium channel gene and overexpression of Cytochrome P450 monooxygenases[6]. Insecticide resistance is a major barrier in the process of vector control. The present study was conducted utilizing the 20th generation of Culex quinquefasciatus, which were reared under the selective pressure of two synthetic pyrethroids: alphacypermethrin and permethrin. The aim

was to investigate the susceptibility status of these pyrethroid-selected strains toward various classes of insecticides, both with and without the application of synergists, as well as to explore the resistance mechanisms involved. This study intends to simulate field conditions characterized by continuous insecticide selection pressure and to evaluate the effectiveness of insecticide rotation. Alphacypermethrin and permethrin were specifically chosen due to their prevalent use in vector control measures, available in the market in forms such as chalks, coils, bed nets, and aerosols, catering to household, public health, and agricultural applications. The incorporation of synergists aimed to assess their preliminary role in metabolic resistance and to determine whether their use enhances the efficacy of insecticides against an already resistant mosquito population.

MATERIALS AND METHODS

Sample collection

Larvae and pupae of the wild population of *Culex quinquefasciatus* were collected from the high drains of the Shivmandir area in November 2021. The samples were then brought to the laboratory and morphologically identified following pictorial identification keys by Das[15] and Tyagi *et al*.,[16].

Insecticides

Alphacypermethrin (technical grade, 97%) and permethrin (technical grade, 98%) were purchased from Heranba Chemicals (Mumbai, India). Insecticide-impregnated papers (5% malathion, 4% DDT, 0.4% dieldrin, 0.05% alphacypermethrin, 0.05% deltamethrin, 0.05% lambdacyhalothrin, 0.75% permethrin, 0.1% bendiocarb, and 0.1% propoxur) were purchased from Vector Control Research Unit, Universiti Sains Malaysia.

Larval Bioassay

A larval bioassay test against alphacypermethrin and permethrin was conducted using the standard procedure by the World Health Organisation[17] to obtain the sub-lethal concentrations. 100ppm stock solution of both insecticides was prepared from the purchased chemicals. From this, five concentrations yielding 10%-90% mortality were prepared to determine the 50% lethal concentration (LC_{50}). Four replicates of 25 late third and early fourth instar larvae were exposed to 100ml

serially diluted five insecticide concentrations in a 250ml glass beaker for 24 hours along with a control setup with no insecticide. Mortality percentages were recorded after 24 hours. Larvae showing no or faint movement when touched were considered dead or moribund. If 5-20% mortality was observed in the control set-up, it was corrected by using Abbott's formula [18] given below:

$$\text{Observed mortality} = \frac{\text{Test mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100$$

Insecticide selection and rearing setup

After obtaining the LC_{50} values, collected larvae were divided into three groups. One group was selected against a 50% sub-lethal concentration of alphacypermethrin and named as 'AS' strain, the second group was selected against a 50% sub-lethal concentration of permethrin and named as 'PS' strain and the third group was susceptible devoid of any insecticide treatment and named as 'S' strain. The selection was carried out by exposing approximately 2000 late third and early fourth instar larvae of each generation to 50% sub-lethal concentration for 24 hours. The larvae that survived after 24 hours of exposure were then shifted to enamel trays (25cm ×30cm ×5cm) filled with tap water. They were provided with powdered fish food till they started pupating. The pupae were separated manually in a beaker and kept inside the mosquito cages (30cm ×30cm ×30cm) for the emergence of adults which were fed with 10% sucrose solution soaked in cotton. After 3-5 days of emergence and successful mating, females were separated and starved for 24 hours before providing an EDTA-treated broiler's blood meal for two hours. The blood meal was again replaced by a 10% sucrose solution. The egg-laying apparatus was set after three days of blood meal. Egg rafts laid were kept individually in separate enamel trays filled with water. The larvae hatched were provided with ground fish food till pupation. The rearing was continued for 20 generations following this procedure.

WHO insecticide susceptibility tube tests

The females from 20th generation aged 3-5days old were exposed to discriminating concentrations (DCs) of one organophosphate (5% malathion), two organochlorines (4% DDT and 0.4% dieldrin), four pyrethroids (0.05%

alphacypermethrin, 0.05% deltamethrin, 0.05% lambdacyhalothrin, and 0.75% permethrin,) and two carbamates (0.1% bendiocarb and 0.1% propoxur) according to the procedure by WHO[19]. WHO[20] has released lesser DCs (0.025% deltamethrin, 0.025% lambdacyhalothrin, and 0.25% permethrin) for *Culex quinquefasciatus*. But since these laboratory strains were under continuous selection pressure the DCs recommended for *Anopheles* were tested. Twenty five non-blood fed females were kept in the 6 holding tubes with green dots containing clean white paper rolled inside the tube fastened with steel spring wire clip for 1 hour (Figure 1).

Fig. 1. Insecticide susceptibility tube test

Any dead or moribund mosquitoes were removed after 1 hour. Silicon oil-treated papers were rolled in yellow dotted tubes and insecticide-impregnated papers were in red dotted exposure tubes fastened with copper spring wire clip. The mosquitoes were then gently transferred to these tubes for 1 hour with regular monitoring at intervals of 10 minutes for recording the knocked-down mosquitoes. After 1 hour of exposure, the mosquitoes were again shifted to green-dotted holding

tubes with no paper this time and provided with a 10% sucrose solution soaked in cotton. Mortality was recorded after 24 hours based upon which susceptibility status was determined.

WHO Synergist Bioassay

To determine the involvement of metabolic enzymes in developing resistance, a synergist bioassay was performed according to the protocol by WHO [19]. Female *Culex quinquefasciatus* were exposed to 4% piperonyl butoxide (PBO) and 10% triphenyl phosphate (TPP) impregnated papers for 1 hour before exposing to insecticide-impregnated papers and rest protocol was like that of susceptibility bioassay test.

Biochemical assays

Thirty non-blood-fed, 3-5days old mosquitoes were homogenized in 200 µl of ice-cold Sodium phosphate buffer (pH 7.2) by Teflon micro-pestle in a 0.5ml microcentrifuge tube followed by centrifugation at 13000 rpm for 2 minutes. The supernatant was used for biochemical assays.

Carboxylesterase assay: It was measured with α-naphthyl acetate (α-NA) and β-naphthyl acetate (β-NA) as a substrate with a minor modification in the method of Van Asperen [21]. The staining agent was Fast Blue BB salt (FBBS). The absorbance (540 nm) was taken by a microplate reader (SPECTROstarnano, BMG Labtech). Blanks contain the same reaction mixture except for the homogenate. Standard curves of α- and β- naphthol were created to estimate the esterase activity.

Cytochrome P450 monooxygenase assay: It was measured by using a substrate namely tetramethyl benzidine (TMBZ) and stained with hydrogen peroxide (H2O2) solution in a microtitre plate well WHO[22]. After 2 hours of incubation, the plate was read at 630 nm in a microplate reader (SPECTROstarnano, BMG Labtech). By using a standard curve of cytochrome C, the activity of CYP450 monooxygenases was calculated.

Glutathione-S-transferase (GST) assay: It was measured with minor modification in the method of Habig et al.,[23]. The homogenate was mixed with 65mM 1-chloro-2,4-dinitrobenzene (CDNB) dissolved in methanol and 10mM of

reduced glutathione (GSH) dissolved in 0.1M phosphate buffer (pH 6.5) and subjected to kinetic assay in SPECTROstarnano, BMG Labtech. The absorbance values were taken for 5 minutes followed by the calculation of GST activity (mM mg protein-1 min-1).

Protein Bioassay: The total protein concentration of the individual mosquito was determined by taking absorbance at 630 nm using SPECTROstarnano, BMG Labtech according to the method of Lowry et al., [24] and compared with Bovine Serum Albumin (BSA) standard curve to nullify size differences and obtain specific enzyme activity.

DNA Extraction

Genomic DNA was extracted from 24 mosquitoes from each strain by High Salt protocol Barik et al., [25] with minor modification. Individual mosquito was homogenized in a 1.5ml microcentrifuge tube using 98µl digestion buffer to which 2µl of 0.2mg/ml proteinase K was added and the mixture was kept in a water bath for incubation at 55°C for at least 2hours. 40 µl of 6M sodium chloride and 157 µl of chloroform were added to the sample and mixed for 20 minutes by gentle shaking followed by centrifugation at 10000rpm for 5 minutes. Only the upper phase, leaving the whitish lower phase, of the supernatant was transferred to the new microcentrifuge tube to which 140 µl of isopropanol was added and put on a shaker for 5 minutes followed by centrifugation at 8000rpm for 15 minutes. A black-colored pellet of DNA could be seen at the bottom of the centrifuge tube. The supernatant was discarded and 150 µl of chilled 70% ethanol was added and subjected to centrifugation at 8000rpm for 5 minutes. The supernatant was discarded and the tube was left to dry. After which, the pellet was dissolved in 20 µl of autoclaved distilled water and stored at -20°C for further use. The ratio of Optical Density at 260nm and 280nm was measured by SPECTROstarnano (BMG Labtech, Germany) to check the purity of the extracted DNA. Genomic DNA with values of a ratio between 1.8 and 2 were considered for kdr genotyping.

Genotyping kdr mutation

The extracted DNA was used to genotype the kdr mutation L1014F in voltage-gated sodium channel gene through allele-specific PCR (AS-PCR) with minor

modifications in the procedure of Martinez-Torres et al., [26] and Sarkar et al., [27]. The primers used were Cgd1 (5'-GTGGAACTTCACCGACTTC-3'), Cgd2 (5'-GCAAGGCTAAGAAAAGGTTAAG-3'), Cgd3 (5'-CCACCGTAGTGATAGGAAATTTA-3') and Cgd4(5'-CCACCGTAGTGATAGGAAATTTT-3'). Three PCR reactions were simultaneously run: Cgd1 and Cgd2 primers were combined in the first reaction to amplify voltage-gated sodium channel gene, Cgd2, and Cgd3 primers in the second reaction to amplify 1014L gene while Cgd2 and Cgd4 primers in the third reaction to amplify 1014F gene. Each reaction was performed in a 25 µl volume mixture of 25ng/µl extracted DNA, 1 µl each of forward and reverse primers, 12.5µl of GoTaq Mastermix (Promega), and 6.5 µl of nuclease-free water. PCR conditions were an initial denaturation at 95°C for 15 minutes, followed by 35 cycles at 94°C for 45seconds, 49°C for 45seconds, 72°C for 45seconds, and a final extension at 72°C for 10 minutes. The PCR products were then analyzed in 2% agarose gel stained with ethidium bromide in a UV Transilluminator (Himedia). The first reaction would yield a product size of 540 base pairs while the second and third reactions a band of 380 base pairs.

Data Analysis

Based upon the mortality percentage against each insecticide calculated, the mosquito population was termed susceptible with ≥ 98% mortality, possible resistant with ≥ 90% but < 98% mortality, and confirmed resistant with < 90%mortality WHO[20]. Abbott's formula was used for the correction of mortality if the mortality in the control group was ≥ 5% but < 20%. The data obtained from larval bioassays and knockdown time (KDT) were analyzed using log-probit analysis Finney *et al*.,[28] in IBM SPSS version 21. The differences in the enzymatic activity between the three strains were checked through analysis of variances (ANOVA).

Results

The larval bioassay of wild *Culex quinquefasciatus* mosquitoes showed LC_{50} of 0.014ppm (0.01-0.018) against alphacypermethrin and 0.035ppm (0.027-0.043) against Permethrin which increased to 1.016ppm (0.784-1.283) and 1.456ppm (1.138-1.846) in 20th generation respectively (Table 1).

Table 1. Sub-lethal concentration (LC_{50}) of different strains of *Culex quinquefasciatus*.

	AS	**PS**
F0	0.014 (0.01-0.018)	0.035 (0.027-0.043)
F20	1.016 (0.784-1.283)	1.456 (1.138-1.846)
SF20	0.007 (0.004-0.013)	0.011 (0.006-0.018)
RR50	145.14	132.36

Data shows 50% lethal concentrations with a confidence interval in the parenthesis. F0- field population, F20- laboratory reared 20th generation, SF20- susceptible strain at 20th generation RR_{50}- Resistance ratio calculated by dividing LC_{50} of selected strains divided by LC_{50} of susceptible strain.

Both AS strain and PS strain at 20th generation of selection showed 0% mortality against 4 synthetic pyrethroids (0.05% alphacypermethrin, 0.05% deltamethrin, 0.05% lambdacyhalothrin, 0.75% permethrin), one organochlorine (4% DDT) and one carbamate (0.1% propoxur), 27% and 40% mortality against 0.1% bendiocarb while 66% and 68% mortality against 0.4% dieldrin indicating resistant status against these insecticides. Possible resistance was seen against 5% malathion in both the AS and PS strains with 90% and 94% mortality respectively (Table 2).

Table 2. Mean Mortality percentages against nine insecticides among three strains of *Culex quinquefasciatus*

INSECTICIDES	**ASF20 (N-100)**		**PSF20 (N-100)**		**SF20 (N-100)**	
	M% ± SE	Status	M% ± SE	Status	M% ± SE	Status
BENDIOCARB	27.00±2.51	R	40.00±1.63	R	100.00±0.00	S
PROPOXUR	0.00±0.00	R	0.00±0.00	R	99.00±1.00	S
ALPHACYPERM-ETHRIN	0.00±0.00	R	0.00±0.00	R	98.00±1.15	S
DELTAMETHRIN	0.00±0.00	R	0.00±0.00	R	99.00±1.00	S
LAMBDACYHAL-OTHRIN	0.00±0.00	R	0.00±0.00	R	98.00±1.15	S
PERMETHRIN	0.00±0.00	R	0.00±0.00	R	98.00±1.15	S

INSECTICIDES	ASF20 (N-100)		PSF20 (N-100)		SF20 (N-100)	
	M% ± SE	Status	M% ± SE	Status	M% ± SE	Status
MALATHION	90.00±2.58	PR	94.00±2.58	PR	100.00±0.00	S
DDT	0.00±0.00	R	0.00±0.00	R	98.00±1.15	S
DIELDRIN	66±2.58	R	68.00±1.63	R	100.00±0.00	S

N- Number of mosquitoes tested, M%- Mortality percentage, S.E- Standard Error, R-Resistant (M%<90), PR- Possible resistance (98%<M%>90%), S- Susceptible (M%≥98%).

Upon treating the strains with synergist PBO and TPP which are cytochrome P450 inhibitor and carboxylesterase inhibitor respectively before exposing them to the insecticides, mortality percentages increased but could not bring full susceptibility. Since, the mortality percentages with synergists followed by insecticides were less than 98% but greater than mortality percentage without synergist, it implies that enzyme-based resistance mechanism accounts only partially for the expression of resistant phenotype (Figure 2).

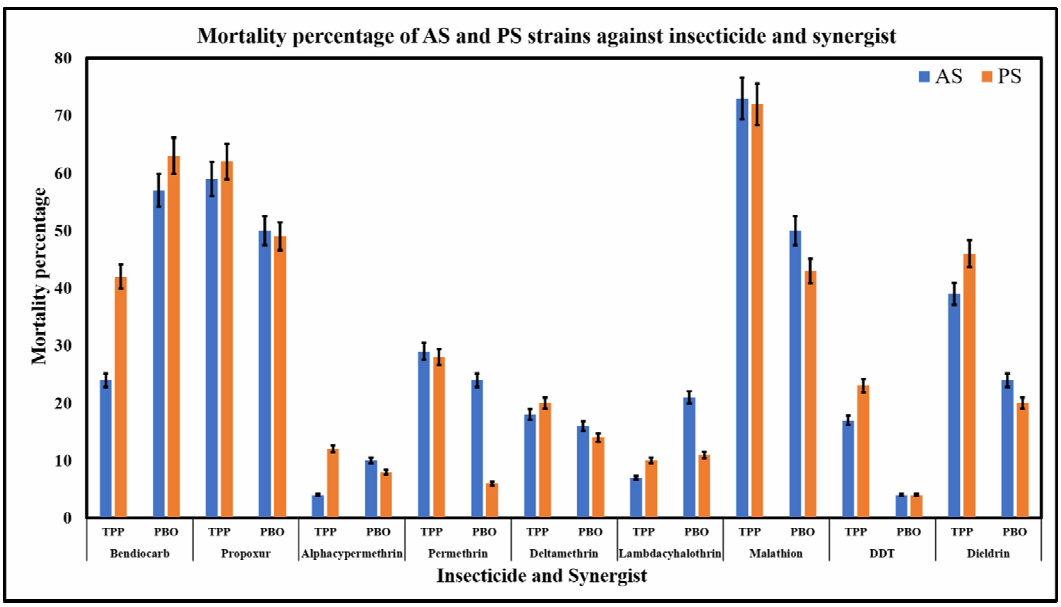

Fig. 2. Graph depicting mean mortality percentages of three strains of *Culex quinquefasciatus* against insecticides and synergists

The duration at which 50% and 90% of mosquitoes were knocked down are designated as KT_{50} and KT_{90} respectively. AS and PS strains showed the lowest KT_{50} against malathion and the highest against dieldrin (Table 3). KDT values for other insecticides could not be calculated as there were no knocked-down mosquitoes within exposure time.

Table 3. Knockdown times (95% confidence interval) of ASF20 and PSF20 strains

INSECTICIDES	STRAINS	KDT50 (CI) (MINUTES)	KDT90 (CI) (MINUTES)	R^2	CHI SQUARE
MALATHION	ASF20 (N-100)	45.62 (39.12-56.37)	106.24 (78.03-201.99)	0.96	1.32
	PSF20 (N-100)	43.70 (40.05-47.85)	64.69 (57.12-80.70)	0.88	4.80
DIELDRIN	ASF20 (N-100)	82.68 (64.90-793.76)	138.03 (87.24-14856.84)	1	1.27
	PSF20 (N-100)	99.83 ($69.36-3.06*10^{10}$)	185.94 ($96.52-3.08*10^{18}$)	0.99	0.39
BENDIOCARB	ASF20 (N-100)	57.99 (48.56-81.53)	134.10 (91.27-344.30)	0.95	1.04
	PSF20 (N-100)	50.46 (42.37-66.87)	128.80 (88.32-301.20)	0.88	2.37

KT_{50}- Duration at which 50% mosquitoes were knocked down, KT_{90}- Duration at which 90% mosquitoes were knocked down, CI- Confidence interval, N- number of mosquitoes tested.

Monooxygenase activity was increased to 2.99-fold and 3.06-fold respectively in AS and PS strains in contrast to the S strain. Carboxylesterase activity and GSTs activity were found not to be significantly different among the selected strains in comparison to that of the susceptible one (Table 4).

Table 4. Enzymatic activity among three strains of *Culex quinquefasciatus*

Strains	***N***	***Monooxygenases (µM EU/mg protein ± SE)***	***Alphaesterases (mM/min/mg protein ± SE)***	***Betaesterases (mM/min/mg protein ± SE)***	***GSTs (µM/min/mg protein ± SE)***
AS	30	38.97±3.37[a]	0.15±0.01[a]	0.011±0.002[a]	1.49±0.54[a]
PS	30	39.89±3.51[a]	0.16±0.02[a]	0.012±0.003[a]	1.67±0.33[a]
S	30	13.03±1.74[b]	0.14±.011[a]	0.010±0.001[a]	1.38±0.11[b]

Values with different alphabets are significant (ANOVA, p≤0.05). SE- Standard Error, N- number of mosquitoes tested. AS- Alphacypermethrin-selected, PS- Permethrin selected, S- susceptible strain.

ASPCR revealed the fixation of L1014F mutation in the selected strains with the total absence wild type genotype (LL) and heterozygous resistant genotype (FS) but only the presence of homozygous resistant (FF). In contrast, this susceptible strain showed 83.34% LL, and 8.34% each of LF and FF respectively (Table 5). The absence of insecticide selection pressure for 20 generations (approximately 1 year 6 months) in susceptible strains still contained the homozygous and heterozygous resistant genotypes.

Table 5. Genotype frequency and allele of three strains of *Culex quinquefasciatus*

STRAINS	**Genotype frequency (%) (N=24)**			**Allele frequency**	
	LL	**LF**	**FF**	**L**	**F**
AS	0	0	100	0	1
PS	0	0	100	0	1
S	83.34	8.34	8.34	0.88	0.13

DISCUSSION

Culex quinquefasciatus not only transmits various mosquito-borne diseases but is a constant source of irritation due to its high abundance owing to the ability to breed in highly polluted water[11] There have been several studies worldwide to monitor the insecticide resistance status in field population of *Culex quinquefasciatus* [1,6,12,13,29,30]. However, fewer to negligible studies have been carried

out in laboratory-selected strains of *Culex quinquefasciatus* [4,31–33]. Hence this study was conducted to evaluate the susceptibility status of two pyrethroids-selected laboratory strains (AS and PS) of *Culex quinquefasciatus* at 20th generation against four classes of insecticides with and without synergists. The study simulates the field condition of *Culex quinquefasciatus* which is under continuous selection pressure and investigates the efficiency of vector control through the use of chemicals. It was observed that AS showed 145-fold resistance and PS showed 132-fold resistance than that of the S strain after 20 generations of selection against alphacypermethrin and permethrin respectively. Both the selected strains showed resistance to synthetic pyrethroids as well as cross-resistance to organophosphates, organochlorines, and carbamates. Moreover, the use of synergists, before exposure to the insecticide, also could not restore the susceptibility in resistant strains though the mean adult mortality rate was slightly higher than that of insecticide alone. It indicates that the detoxification by the metabolic enzyme is not the major insecticide resistance mechanism involved. This finding contrasts with other studies where a significant increase in the mortality rate and restoration of susceptibility was observed[11,34]. On the other hand, a study by Lucas *et al*.,[35] also reported the partial involvement of metabolic enzymes in the resistance status of *Culex quinquefasciatus*. Biochemical assays showed higher activities of monooxygenases in selected strains. Moreover, fixation of kdr mutation L1014F was found in selected strains. Overexpression of cytochrome P450s and presence of L1014F mutation has been reported in various previous studies[4,13,31,33,35]. Detoxifying enzymes can act individually or synergistically, sequentially metabolizing the same or different insecticides and often work together with target-site mutations which leads to soaring resistance levels along with cross-resistance. This might be the reason for the cross-resistance observed in our study. Additionally, pyrethroid resistance may have pleiotropic effects, influencing multiple biochemical pathways, including those involved in organophosphates, organochlorines and carbamate resistance. Moreover, repeated exposure to pyrethroids may have selected mosquitoes with pre-existing resistance to other insecticides. This study highlights the need for the development of novel insecticides with different modes of action.

CONCLUSIONS

This study provides valuable insight into the importance of monitoring insecticide susceptibility status before the application of a particular insecticide to

know its efficiency in controlling the vector. With the rise in resistance against insecticides, integrated vector management methods for the control of mosquito vectors must be encouraged such as the use of biological control agents, breeding habitat destruction and most importantly mass awareness among the public about sanitation and vector-borne diseases. Insecticide use should be minimized and kept for emergency outbreaks so that the epidemic outbreak can be controlled at the earliest. For successful vector control, coordinated works of various organizations and individuals are required which includes researchers, chemical industries, control agencies, national and international governments, and local populations, and successful implementation at the ground level.

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Scientist's Biobibliography

DR T. RAMACHANDRA RAO: AN EXTRAORDINARY MEDICAL ARTHROPODOLOGIST AND MALARIAOLOGIST

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ABSTRACT

Biobibliography of Dr T. Ramachandra Rao, an arthropodologist nonpareil and an internationally repured malariologist, especially for his works on the control of malaria through vectors in India, is presented for the first time ever in the annals of science.

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Part - 1

BIOGRAPHY

Dr Thammajirao Ramachandra Rao (1907-1984) was an awe-inspiring personality known for his dynamism, huge energy and relentless perseverance in the realms of malaria control in India, and for the highly popular monograph '*Anopheles of India*', his *magnum opus*, which still continues to be a powerful reference work for every serious malaria research worker in India.

"Sow a thought, and you reap an act;
Sow an act, and you reap a habit;
Sow a habit, and you reap a character;
Sow a character, and you reap a destiny."

Dr T. Ramachandra Rao (1907-1984)

Popularly known as TRR, Dr T. R. Rao was an eminent Entomologist who took charge of Virus Research Centre (VRC, presently National Institute of Virology) in 1961. Dr Rao was awarded D.Sc. degree from University of Calcutta in 1951 and was working with Bombay Public Health Department (Maharashtra Govt) before joining VRC. During 1953-55, Dr Rao spent two years on deputation with the Institute to train scientists of the newly established VRC on medically important arthropods especially mosquitoes. The mandate of establishing VRC was investigation of arthropod borne viruses in India as part of the global initiative of Rockefeller foundation. Dr Rao worked as a resource person for Entomology division of VRC till 1955 and during his brief tenure he trained scientists and technical staff in mosquito taxonomy that helped in the discovery of many new species of mosquitoes. He helped in grooming several young scientists to become eminent entomologists like Dr PK Rajagopalan (Director VCRC), Dr HR Bhat etc. Dr Rao returned to his parent organization, *i.e*., Bombay Public Health Department and resumed his work on malaria and filariasis control as a Deputy

Director. As a state entomologist, his major contribution was the studies on malaria vectors and control of rural malaria.

Dr T.R. Rao emerged as one of the hard-working all-round specialist medical arthropodologists in independent India. In the pre-independent India, most of the notable contributions were made by scientists such as Muirhead Thomson in Assam, Robert Knowles Ronald Senior-White and colleagues in Orissa (now Odisha), Mandayam Osuri Tirunarayana Iyengar and R.N. Sen in Bengal, Russell and Ramachandra Rao in South India, D.K. Viswanathan and Ramachandra Rao in the old Bombay State, and B Ananthasamy Rao in the erstwhile Mysore State. They contributed considerably to our understanding of the bionomics and ecology of vectors such as *Anopheles culicifacies*, *A. stephensi*, *A. minimus*, *A. fluviatilis*, *A. philippinensis*, and *A. sundaicus* (Diptera: Culicidae). In India, the time between 1930 and 1945 could be regarded as the golden era of studies on the bionomics and ecology of malaria vectors. The work by the Malaria Institute of India under the leadership of Gordon Covell needs to be remembered in this context. During the late 1930s, Russell and Ramachandra Rao used pyrethrum as a space-spray against anophelines in the malaria-affected areas of Pattukkottai (Thanjavur district, Tamil Nadu) where irrigation practices were defective. Pyrethrum sprays were used within human residences against the adult *A. culicifacies*. Spraying of pyrethrum extracts as mist inside human dwellings during daytime killed the resting adult mosquitoes. They extended their work to North Kanara district of the old Bombay State in 1945, a high malaria-prone area. Then DDT appeared on the scene and revolutionized malaria management when it was sprayed on the walls and ceilings of human dwellings because the vector mosquito rested there after an infected blood meal. This method was successfully used to protect civilian populations by Viswanathan and Ramachandra Rao in North Kanara in 1945 and Senior-White in Odisha. Almost simultaneously, B.A. Rao and his team trialled it successfully in other parts of the country. In 1946, Viswanathan and Ramachandra Rao launched one of the largest malaria control projects in the rural India, seeking to protect over a million humans in the North Kanara and Dharwar Districts in the erstwhile Bombay State, and it proved a crashing success. DDT sprays were ineffective here, because the vector *A. fluviatilis* usually rested outside human residences.

Impressed by his meticulous work on mosquitoes, he was offered the directorship of Virus Research Centre (VRC) of the Indian Council of Medical

Research and Rockefeller Foundation in 1961, which he happily accepted. Dr Rao worked as Director of VRC till 1970 and joined the WHO funded mosquito genome project (GCMU) as per ICMR directive. At VRC, Dr Rao's interest involved epidemiology of vector borne diseases with special reference to arboviruses. During the tenure as a director, worked on the major vector-borne viruses, *i.e*., dengue, chikungunya, Japanese encephalitis and Kyasanur Forest Disease (KFD) virus etc., helping the government to tide over outbreaks by timely diagnostic support and vector management. In addition, several new viruses from mosquitoes, ticks and sand flies were isolated which were new to science and developed diagnostics for rapid diagnosis. He was also instrumental in the development of a KFD vaccine which saved numerous lives in the KFD endemic areas. With his indefatigable spirit, inspiring and dynamic leadership, he successfully guided and expanded development activities at VRC making it a world class research laboratory for arboviral research with highly trained personnel and infrastructure. Two field units one at Bangalore and the other at the Stanley Medical college Madras were established to cater the needs of the two states. VRC has achieved the status of WHO's collaborating centre for arbovirus and haemorrhagic fever reference and research for Southeast Asia during Dr Rao's tenure.

A monograph entitled, "*Anophelines of India*" is a classic contribution by Dr Rao to the malariologists across the country. This contribution represents the mission and dedication of Dr Rao's life as he spent his entire life in the study of mosquitoes especially Anopheles mosquitoes during his initial phase and later on mosquitoes of arboviral importance. Apart from mosquitoes, he has tremendous knowledge in Acarology especially on ticks and mites. Eminent scientists like Dr HR Bhat and Dr SM Kulkarni were his students who became leading taxonomists for ticks and mites respectively in the country. A brief overview of Dr Rao's achievements at VRC is given in the ensuing section.

1. ***Dr T.R. Rao as the Director, Virus Research Centre*** *(now National Institute of Virology):* Since the mandate of the Institute was identification of arthropod vectors and arboviruses, Dr Rao initiated hematophagous arthropod surveys across the country to study virus transmission and detection of viruses during inter-epidemic periods giving special importance to ecological studies to understand the natural cycle of arboviruses. The hematophagous arthropod survey in the Himalayan

Region spanning from Kashmir to the erstwhile NEFA (present Arunachal Pradesh) generated huge data on blood-sucking arthropods along with vertebrate reservoir hosts of arboviruses, *viz*., birds, mammals, and reptiles. During the survey, several new species of arthropods [one species of mosquito, two species of sand flies, 14 species of lice, two species of fleas, three species of bugs, 18 species of ticks and 64 species of mites were discovered and reported. Also, one new species of rodent and a new species of bird were described for the first time. A zoology museum comprising 52 species of rodents, 342 species of birds, 22 species of bats and a huge collection of arthropods [mosquitoes (203 spp.), ticks (52spp.), fleas (35 spp.), mites (50spp.) and sandflies (15spp.)] was established at VRC for academic and research purposes. A well equipped laboratory was established at VRC during his reign and Dr Rao introduced experimental studies in the laboratory to substantiate the vector potential of the arthropods. To study tick borne viruses, laboratory colonies of 14 medically important tick species were established at VRC. Similarly, mosquito colony of important mosquito species, *i.e*., *Aedes aegypti, Ae. albopictus, Culex tritaeniorhynchus, Cx fatigans, Anopheles stephensi,* etc., were also established and virus vector interaction studies were initiated. Phenomenal contributions towards the understanding of major arboviruses of the country, *viz*., Kyasanur Forest Disease virus (KFDV), Japanese encephalitis virus (JEV), West Nile virus (WNV), Chandipura virus (CHPV), (CCHFV), dengue virus (DENV), chikungunya virus (CHIKV), etc., were made during his tenure.

2. ***KFD studies:*** Major contribution as the Director of VRC, was the field biology studies on KFDV, which causes fatal infection in monkeys and occasionally in humans in Shimoga district of Karnataka. Comprehensive field studies unearthed valuable information about the virus, *i.e*., its maintenance in nature including vectors, reservoir hosts, amplifying hosts and other ecological factors etc. Studies revealed the involvement of 16 species of ticks in KFDV transmission and the role of small forest mammals such as rats, mice, squirrels, insectivorous and frugivorous bats, shrews and birds in the maintenance of virus in nature. Colonies of important KFD vector ticks were maintained and their vector potential as well as transovarial transmission was demonstrated experimentally.

3. ***KFD vaccine research:*** Dr Rao envisioned the need to have a vaccine against KFD virus to contain the recurring outbreaks in Karnataka. Under his eminent leadership, studies were initiated on a war footing to develop formalin inactivated and live attenuated candidate vaccines at VRC. Though both the vaccine candidates elicited immune response, the live attenuated vaccine yield was low compared to the killed vaccine. Initially a formalin inactivated mouse brain based vaccine was developed field trials were done, Subsequent field studies gave good immune response among the vaccinees and no untoward incident was reported. The mouse brain inactivated virus was later on replaced with cell culture based vaccine and were produced by Karnataka Government for routine immunization in endemic areas.

4. ***JE studies:*** Extensive studies to understand the natural cycle of JEV that caused large-scale outbreaks in different parts of India since 1955 were also undertaken. Field studies revealed 19 vector mosquito species responsible for the virus transmission, as well as the reservoir and amplifying hosts instrumental for maintenance and dispersal of the virus. In-depth studies, *i.e*., vector seasonality and disease occurrence, virus isolation from wild caught mosquitoes, humans and sentinel hosts, etc. have been carried out during outbreaks reported from different parts of the country. Demonstration of absence of viremia in cattle is a classic piece of work.

5. ***Dengue virus studies:*** At the time of taking charge as Director, dengue was not a major concern in the country. However, dengue cases increased substantially especially in the southern parts of India and VRC in collaboration with scientists at the CMC Vellore carried out extensive research. Major focus was given on the vector control, *i.e., Aedes aegypti* control. All the four serotypes were isolated from both human serum and *Aedes aegypti*. VRC played an important role in the management of dengue in India with investigation of outbreaks, diagnosis and control.

6. ***Chikungunya outbreak in West Bengal, Madras, Maharashtra and other south Indian cities:*** Dr Rao played a pivotal role in the detection and containment of the eitiological agent which struck for the first time in India. The 1963 outbreak as hemorrhagic fever outbreak was massive which killed more than 200 people in Calcutta was investigated by VRC

along with the School of Tropical Medicine Calcutta. The etiological agent and the vector responsible for transmission were identified as Chikungunya and *Aedes aegypti* respectively. Virus isolation from mosquitoes and human sera confirmed the etiology of the outbreak and control measures were initiated which contained the outbreak in a short span. Subsequent outbreaks in other parts of the country, *i.e*., Nagpur, Madras, Vellore, Rajahmundry, Vishakhapatnam etc were also massive and investigated by VRC with the local support from CMC Vellore. Dr Rao initiated a number of entomological, serological and ecological studies to unravel the mystery of chikungunya virus transmission and maintenance in nature.

7. ***Isolation of novel arboviruses from vectors and humans****:* During the KFD and JE outbreak investigations, Dr Rao gave due importance to look for other etiological agents in the vectors and human sera collected from the outbreak area. During his period, more than 30 arboviruses and several other viruses were isolated from vectors, humans, birds and other animals. Chandipura virus, an encephalitic virus that causes high mortality in children was first isolated from patients with dengue-chikungunya like illness in 1965 from Nagpur area. Extensive work revealed the vectors involved in the maintenance and transmission of the virus by virus isolation from *Phlebotomus* and *Sergentomyia* sand flies. Vector competence of several important mosquitoes to the virus was also studied. Though mosquitoes including *Aedes aegypti* transmitted the virus experimentally, no isolation of the virus from the mosquito collected from the outbreak area could be obtained suggestive of its negative role in virus transmission. The virus was responsible for a large outbreak among children with more than 300 fatal cases in 2003-4 in the Central India. The other viruses of importance isolated during the period are Kaisodi, Ganjam, Wanowrie, Dhori, Chittoor, Balagodu, Arkonum, Satuperi, Minnal, Umbre, Palyam, Venkatapuram etc., to name a few. Serological investigations were initiated with retrospective samples as well as fresh samples to determine the existence of these viruses in the country. Extensive entomological and virological studies across the country revealed the epidemiology of West Nile virus In India. WNV was isolated from mosquitoes, bats and humans from different parts of India.

Seroprevlence studies have shown antibodies to the virus among humans across the country.

8. ***Establishment of arthropod cell lines for arboviral research:*** Another feather in the cap of Dr Rao was the establishment of mosquito and tick cell lines for arbovirus research at VRC. World's first mosquito cell lines (was established from *Aedes aegypti* and *Aedes albopictus* mosquitoes by Dr KRP Singh under Dr Rao's supervision in 1967. The establishment of cell lines from hematophagous arthropods eased studies on virus isolation and virus vector interaction. Dr Singh's ATC-15 cell line developed from *Aedes albopictus* mosquitoes garnered worldwide acceptability among researchers due to its broad spectrum susceptibility to arboviruses as it supported replication of more than 50 arboviruses. A clonal population of the cell line, C6/36, developed by Dr. Akira Igarashi in 1978 has generated tremendous interest among virologists and is the most widely used cell line for dengue virus studies worldwide. Subsequently, several cell lines from important vector mosquitoes, *viz*., *Culex tritaeniorhynchus, Cx. bitaeniorhynchus, Aedes vittatus, Ae. novalbopictus,* etc. were developed. Three tick cell lines were also developed to study tick-borne viruses.

9. ***Awards and Honours:*** Dr Ramachandra Rao was conferred TS Narayana Rao Oration Award by ICMR (1974); Sisir Kumar Mitra Lecture Award by INSA (1978); and Sharada Devi Paul Memorial Oration by National Institute of Virology. He was President, Zoology Section, Indian Science Congress (1972); Fellow, National Academy of Medical Sciences (India), National Society of Communicable Diseases (India), Royal Society of Tropical Medicine and Hygiene; and Honorary Member, American Society of Tropical Medicine and Hygiene.

Dr T. R. Rao will continue to shine forever in the world of malaria and mosquito research. His life and works continue to motivate thousands of scholars all over country and beyond, just like the Sun which brings shine to all on our planet.

"If you want to shine like a Sun,
first burn like a Sun."

– *Dr A.P.J. Abdul Kalam*

PS:- *Prof. Dr B.K. Tyagi memorizes his momentous meeting with Dr T.R. Rao:*

> "I have had the rare opportunity to interact with him, in the office of Dr V.P. Sharma, Director, Malaria Research Centre, Delhi in March, 1981. He sermonized for me," *Dr Tragi, think how you can control malaria which will be your biggest contribution to the country*." The message is imprinted indelibly on my mind, and I left no stone unturned in this regard during past four decades of my research on malaria culminating with my book on "Desert Malaria" published in 2023 (Tyagi, B.K., 2023. *Desert Malaria: An Emerging Malaria Paradigm and Its Global Impact on Disease Elimination*. Springer-Nature, Singapore, 416 pp.)! My journey is not finished yet….!!

"The woods are lovely, dark and deep
But I have promises to keep,
And, miles to go before I sleep,
And, miles to go before I sleep."

– *Robert Frost*

Part - II

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★ ★ ★ ★ ★

Book Review

DESERT MALARIA: AN EMERGING MALARIA PARADIGM AND ITS GLOBAL IMPACT ON DISEASE ELIMINATION

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Senior Scientist (Retired)
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Received : 30th October, 2024

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My searches for a suitable reference on desert malaria landed on one which turned out to be a book, **'Desert Malaria: An Emerging Malaria Paradigm and Its Global Impact on Disease Elimination by B.K. Tyagi, Springer 2023, pp. xxi + 416'**. It is indeed a treatise giving full account of 'Desert Malaria', an emerging paradigm of malaria transmission in the inimical and inhospitable desert, xeric/arid environments, which remained clear neglect by the control programme for long.

The book encompasses 24 chapters, each dwell on the subject in its entirety providing added knowledge on the dimensions in understanding lesser known 'Desert Ecosystem', helping prioritize strengthening health systems to meet future challenges in public health. Deserts are rapidly transforming on account of climate

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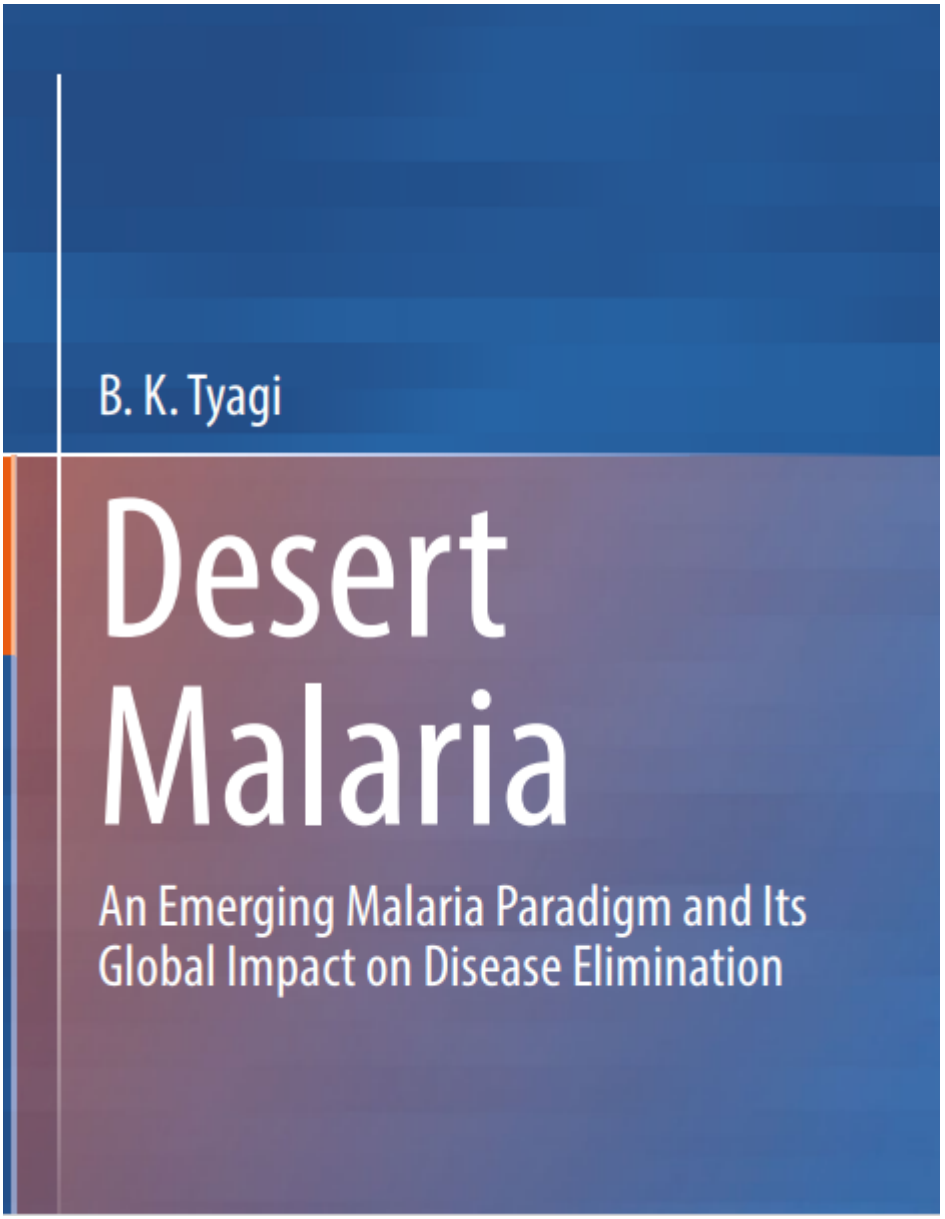
change and increased anthropogenic activities lending to increased mosquito menace and associated disease transmission risk.



Desert Malaria: An Emerging Malaria Paradigm and Its Global Impact on Disease Elimination by B.K. Tyagi, Springer 2023, pp. xxi + 416

The message is sound and clear that disease burden is manyfold than estimated calling for renewed efforts formulating appropriate policy if malaria elimination is to be realized by target date of 2030. Disease epidemiology is fast changing driven by population explosion, urbanization, industrialization, expanding infrastructure including highway network and canal irrigation system lending to establishment of invasive vector species such as *Anopheles stephensi* and *Aedes aegypti,* which are far more efficient in disease transmission of malaria and arboviruses such as dengue respectively. It is a clarion call for programme officials to prioritize desert regions for allocation of resources to meet the growing healthcare needs in keeping with population growth.

This book is indeed thought provoking and would be a useful document for programme and policy managers, research establishment and stakeholders helping formulate interventions to combat mosquito menace and disease burden. It would certainly be good desk book for faculty and students alike to keep abreast of the changing disease ecology and invigorating newer intervention tools.

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Senior Scientist (Retired)
ICMR – National Institute of Malaria Research, New Delhi

★ ★ ★ ★ ★

Book Review

MOSQUITO-BORNE DISEASES IN INDIA

(Current status and prospects of elimination)

Authored by Vas Dev and Vijay Veer

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This book comes at a time when India is strongly surging ahead to eliminate a few deadly and/or debilitating mosquito-borne diseases (MBDs) by 2030. Keeping this in mind, the book, a vade mecum for a serious researcher in India, deals with

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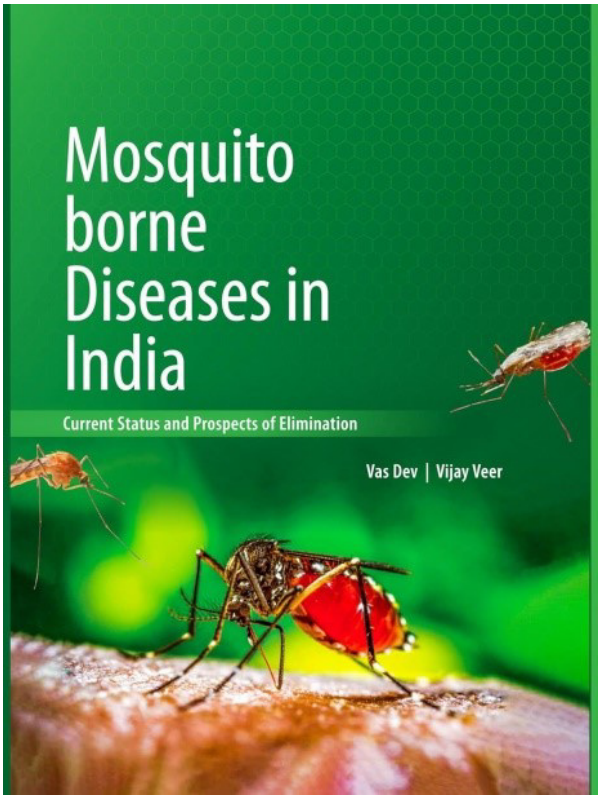

all the major MBDs, viz., malaria, lymphatic filariasis, Japanese encephalitis, dengue, chikungunya and zika.

This book has ten chapters, under three Sections, all within 134 pages. The first two chapters describe mosquito generalities, the next six chapters inform *in extenso* about each disease. especially, epidemiology, control, surveillance, diagnosis and treatment. Narration of these diseases is made in a lucid and simple language which the readers will surely appreciate.

What is more interesting is that authors have ventured to bring on surface several of the challenges that lie ahead of achieving the target. An array of 'frequently asked questions' session adds value to the book since even basic information is essential for understanding the mosquito vector and the diseases they transmit. To make our understanding better authors have discussed the SWOT analysis for eliminable MBDs.

The is rich in text, tables and figures, most of which elegantly colourful. The book should be consulted by one and all engaged in the research related to mosquito-borne diseases. The book (ISBN-10: 936028808X, ISBN-13: 978-9360288082), published in the year 2024, can be purchased from the publisher: M/S Bishen Singh Mahendra Pal Singh, 23 A New Connaught Place, Chakrata Road, Dehradun - 248001, Uttarakhand, India; Phone:+91 135 2715748; Fax:+91 135 2715107; Email: bsmpsbooks@gmail.com, bsmps@bsmpsbooks.com; Website: bsmpsbooks.com

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In Memoriam

OBITUARY

RENOWNED SCIENTIST (PROF KARAMJIT SINGH RAI) BIDS GOODBYE TO THE MORTAL WORLD

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Received : 24th November, 2024
Accepted : 26th November, 2024

Professor (Dr.) Karamjit Singh Rai (born 1931, village Moranwali, district Hoshiarpur, Panjab, India), an alumnus of Panjab University, Chandigarh, received his doctorate degree from the University of Chicago in 1960, and served as Professor of Biological Sciences at the University of Notre Dame, Indiana (a reckoned institution for academic excellence), Indiana, USA for the period 1962–1999. During this tenure spanning four decades, he made laudable contributions in understanding mosquito genetics and cytogenetics of *Aedes aegypti* (an important disease vector of yellow fever, dengue and Zika), and mentored host of students including those from India towards doctorate degree as well as postdoctoral trainees.

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Karamjit Singh Rai
(1931 – 2024)

He was a professor of eminence and guide par excellence in his chosen discipline of research evidenced by scholarly research publications in international journals of repute. His research efforts provided insights in understanding mosquito genomes and mechanisms of evolution and speciation. Taking cognizance of his expertise, he was invited to establish Department of Biology at the Guru Nanak Dev University (formerly Khalsa College), Amritsar (Panjab, India), and later he rendered his services as honorary Professor. His expertise was equally sought by the United Nations agencies including World Health Organization, Geneva, International Atomic Energy Agency, Vienna and served as Advisor to the Government of Sri Lanka, Brazil, Thailand and India.

No love lost for his motherland, he returned to his roots after nearly forty years of stay in the United States and founded "S. Jaswant Singh Rai Memorial Public Charitable Trust" in memory of his father and got actively involved in philanthropic activities making generous donations to educational and healthcare establishments helping expand infrastructure reaching out to those most in need, and hosting invited lectures, awards and fellowships to the deserving students. He was indeed a 'Gur Sikh Gentleman' maintaining Sikh identity, practices and values all throughout his journey of life.

He breathed his last on 5th March 2024 at 93 years of age after a brief illness. He survived by his wife, four sons and a daughter; all benefitted by best of education in the United States presently holding managerial positions in US companies with global presence. He fully lived his life and will be remembered for long for his notable contributions to science and humanity, benevolence and above all forgiving nature and humility. May his soul rest in peace.

This write up was contributed by Dr. Vas Dev in loving memory of Prof. Rai under whom he had received his doctorate degree from the University of Notre Dame, Indiana way back in 1983.

★ ★ ★ ★ ★

Editors' Gratitude to Reviewers

The Editors are thankful to the Referees and the Members of Editorial Board (*vide infra*) for their continued support and guidance in finalizing the manuscripts for the Vol. 4 (Nos. 1 & 2) published on June 1 and December 1, 2024. We sincerely continue to solicit their guidance and support in our future ventures as well.

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Suggestions to Authors

Introduction

The Journal invites Original articles, Short communications, Editorials, Review articles, and other type of scientific information in the field of Medical Arthropodology & Public Health from prospective authors worldwide. At present, the journal does not take any charge for submission, processing, publication of manuscripts, and copy/supply of pdf version of the research paper published.

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Perspective	2000	-	10
Original Article	3500	8	35
Review/Update Article	4500	8	45
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Letter to Editor	500	2	3

Manuscripts submitted to the ***Journal of Medical Arthropodology & Public Health*** (*J Med Arthropodol & Public Health*) should not have been published previously or be under simultaneous consideration for publication by any other journal. Any violation of this will lead to a retraction of the published article by the Journal and any other actions as deemed necessary by the Editorial Board. All manuscripts including invited articles will be peer-reviewed. Accepted articles will be edited to the Journal's style. Accepted submissions will become the permanent

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The contribution of each author is to be mentioned, on the Author Certificate in all multi-author research papers under the following headings: Study Concept, Drafting & Manuscript Revision, Statistical Analysis, Study Supervision.

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Examples of reference style

(i) *Standard Journal Articles –*

(a) *Single author:* Cowan G. Rickettsial diseases: the typhus group of fevers – a review. Postgrad Med J. 2000; 76 (895): 269-72.

(b) *Up to six authors:* Schwartz J, Coull B, Laden F, Ryan L. The effect of dose and timing of dose on the association between airborne particles and survival. Environ Health Perspect. 2008; 116: 64-9.

(c) *More than six authors:* Baselga J, Campone M, Piccart M, et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med. 2012; 366: 520-29.

(ii) *Organization as Author –*

National Vector Borne Disease Control Programme. Dengue/Dengue Haemorrhagic Fever. Delhi: National Vector Borne Disease Control Programme; c2005-2018. Available from:
http://www.nvbdcp.gov.in/dengue5.html, accessed on March 26, 2018.

(iii) *Epub ahead of print with DOI –*

Slamon D, Neven P, Chia S, et al. Overall survival with ribociclib plus fulvestrant in advanced breast cancer. N Engl J Med. 2019; published online Dec 22. DOI:10.1056/NEJMoa1911149.

For further referencing in Vancouver style, authors are advised to go through the following document link–
https://www.imperial.ac.uk/media/imperial-college/administration-and-support-services/library/public/vancouver.pdf

Tables

Tables should be typed serially numbered in Arabic numerals (e.g. Table 1, Table 2) with a short title specifying the contents. Horizontal lines should not be used in the body of the table except between a column heading and its sub-headings. Vertical lines should not be used in the table. Tables should be concise and restricted to a page in length and should be self-explanatory. The data presented should not be duplicated in the text.

Figures/Photographs/ Illustrations

Colour images may be submitted but must be in good quality of production. Illustrations should have at least 300 × 300 dpi resolutions and be clear enough. Photographs/illustrations may be submitted as 'JPEG', or 'TIFF' files. Line art drawing must have a minimum resolution of 1200 dpi. Borrowed photographs or

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Units: All measurements must be in metric units, preferably with corresponding SI units in parentheses. No periods, no plural form should be used (e.g. '10 cm' not '10 cms.').

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These should not be included in the references list but may be described in the text. The author(s) must give the full name, affiliation and the date of communication, and whether it was in oral or written (letter, fax, email) form. A signed statement of permission of the personal communication or as a source for unpublished data should be forwarded along with the submission.

Ethics

Any submission involving human subjects should have been conducted with informed consent by the subjects and of approval by the institutional ethics committee.

Keywords

Not more than 3-6 Keywords in alphabetical order should be mentioned in the abstract section of the following article categories: Review Articles, Original Articles and Short Communications.

No keywords are required for Editorials, Perspectives, and Letters to the Editor.

Acknowledgements

All individuals who were directly involved with the study but do not qualify to be authors should be acknowledged. Consent should however be taken from these

individuals prior to including their names. People who have provided only secretarial, clerical or technical help and whose contribution was limited to their routine job profile should not be included in the acknowledgement.

The Chief Editor shall have the final decision-making power to accept or reject a submission and also reserve the right to adjust the style to certain standards of uniformity and suitability of the journal.

All correspondence regarding manuscripts and inquiries, if any, should be made to the Executive Editor Dr Rina Tilak [***email**:* rinatilak@hotmail.com], with a cc. to Prof. Dr B.K. Tyagi [***email:*** abktyagi@gmail.com].

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Request for contributing manuscripts for the next issue scheduled to be published on June 1, 2025

The power of scientific research lies in its ability to transform people's lives. Helping colleagues, peers and the wider general public to get a better understanding of your research and the impact that it can have on society is a win for everyone involved. Promoting your research helps it reach a wider audience in the arena of your research interests, which could lead to future collaboration and further academic opportunities, such as invites to conferences and commissioned articles.

Journal of Medical Arthropodology & Public Health, an open access journal, is an official organ of SOCIETY OF MEDICAL ARTHROPODOLOGY (SOMA; www.soma16.org), published semiannually on June 1 and December 1. It is general policy that all manuscripts are critically peer-reviewed. It focuses on medically important arthropods' parasitological and pathological significance in public and veterinary health as well as their hazardous, pestilent and/or vectorial behaviour to mediate a large number of deadly and/or debilitating diseases, besides poisonous bites and stings, allergies etc., on one hand, and their pharmacological, nutritional, medicinal, biotechnological and bioengineering utilities for direct human benefit, on the other.

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Vol. 4, No. 2, of *Journal of Medical Arthropodology & Public Health* (December 1, 2024) was published on date. We take this opportunity to request the scientific fraternity having research interests in medically important arthropods (e.g., insects, arachnids, centipedes, millipedes, crustaceans etc.) to submit their research manuscripts for consideration of publication in the Vol. 5, No. 1 (June 1, 2025) on or before March 30, 2025. For SUGGESTIONS TO AUTHORS please see either this issue or the SOMA website, www.soma16.org.

Expecting your kind understanding and cooperation in this regard, and looking forward to receiving your manuscripts before very long, we remain, with best wishes,

Cordially yours,

Prof. Dr B.K. Tyagi **&** **Dr Rina Tilak**
Chief Editor Exec. Editor

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1. Name of the periodical	**:**	**Journal of Medical Arthropodology & Public Health** **ISSN: 2583-6455 (Online)**
2. Published by the Society	**:**	SOCIETY OF MEDICAL ARTHROPODOLOGY
3. Registration Number	**:**	103 of 2017 (Registrar of societies, Hyderabad; dt. Mar 1, 2017)
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5. Address of Publication	**:**	Department of Zoology, University College of Science, Osmania University Hyderabad, Telangana-500007 India
6. Frequency	**:**	Semiannual (Commencing w.e.f. June 1, 2021)
7. Language	**:**	English
8. Date of Declaration	**:**	1st December, 2024

We solemnly declare that above information is true to the best of our knowledge.

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