MEJAST

Middle East Journal of Applied Science & Technology (MEJAST)

Volume 8, Issue 3, Pages 82-94, July-September 2025

Biological control of *Aedes* larvae using native larvivorous fish *Colisa labiosus* in selected areas of Hinthada District, Ayeyarwady Region

Maung Maung Mya[1*], Min Zaw Latt[2], Chit Thet Nwe[1], Zar Zar Aung[1], Thu Zar Nyein Mu[1], Yee Yee Myint[1] & Sein Thaung[1]

[1]Medical Entomology Research Division, Department of Medical Research, Myanmar. [2]Zoology Department, Yangon University, Myanmar. Corresponding Author (Maung Maung Mya) Email: dr.mgmgmya@gmail.com[*]

DOI: https://doi.org/10.46431/MEJAST.2025.8306

Article Received: 17 May 2025 | *Article Accepted: 26 July 2025* | *Article Published: 29 July 2025*

ABSTRACT

Dengue hemorrhagic fever and Dengue fever are harmful to human mostly children and they are a public health problem in Myanmar. An intervention study was conducted in Ywa-Thit village Hinthada Township as test village and Tha-Phan-Pin Ward Ingapu Township as non-intervention village. Six months study was conduct from May to October 2023 in Hinthada District Ayeyarwady Region. Fifty households each were randomly selected for *Ae. aegypti* larvae detection in water storage containers and Larval and Pupal Indices, Kye-containers and Key-premises were measured in both areas before and after interventions. Before intervention larva consuming rate of larvivorus fishes were tested against laboratory reared 3rd and 4th instar *Aedes* larvae in different water volumes. One inch length of *Colisa labiosus* found maximum number of larvae (1553±38) consumed within 24 hours was used to control *Aedes* larvae in water storage containers in Ywa-Thit village. In Ywa-Thit village 2 fishes each were put in major and one to two fish each was put in minor and miscellaneous containers. Result revealed that after intervention larva positive containers were significantly reduce from 64.19%, 35.16% and 66.23% to 0%, 0.89% and 1.61% respectively in major, Minor and miscellaneous containers in intervention area of Ywa-Thit village. Larval-Indices as Container-Index (CI), House-Index (HI) and Bretuae-Index (BI) were reduced from 51.75%, 78% and 296 to 0.76%, 2% and 4. Key-containers and Key-premises were also reduced from 24, 14 to 0, 0 respectively in Ywa-Thit village. Pupal-Index (Pupae/house, Pupae/container, Pupae/person and Pupae/child) were also reduced from 3.9, 0.68, 0.96 and 4.24 to 0 each in Ywa-Thit village (P=0.001). Although after-intervention, in nan-intervention Tha-Phan-Pin Ward, container positivity, larval and pupal indices, Key-containers and Key-premises were found to be increased and Key-containers and Key-premises were found increased to 62% and 34%. Native larvivorous fish *Colisa labiosus* is a very effective and suitable control tool for *Aedes* larvae in water storage containers in Hinthada Township. In conclusion, C*olisa labiosus* acts as a potential larvivorous effect of *Aedes* larvae and an effective biological control agent, eco-friendly, non-resistance and cost-effective, to be considered as a potent natural larvicidal agent to control *Aedes* larvae in the community.

Keywords: *Aedes aegypti*; Biological; *Colisa labiosus*; Consuming Rate; Containers; Fish; Larval Indices; Larvae; Larvivorous; Major; Minor; Miscellaneous; Pupal Indices; Water Storage Containers.

1. Introduction

Mosquito borne diseases are major public health problem in Myanmar and mosquitoes are the seriously harmful to human and fetal insects in tropical and subtropical regions in the world [1] due to the transmission of various diseases, such as the West Nile virus, filariasis, dengue, chikungunya, Japanese encephalitis, and malaria in humans [2]. *Aedes aegypti* L. (Diptera: Culicidae) is a primary vector of Dengue fever (DF) and Dengue haemorrhagic fever (DHF), chikungunya, yellow fever, and Zika virus [2]. *Culex quinquefasciatus* is a primary vector of Filariasis, *Culex tritaeniorhynchus* is a main vector of Japanese encephalitis, and *Anopheles dirus* and *An. minimus* are the main vector of malaria in Myanmar [3,4,5,6]. Moreover, *Aedes* mosquitoes are listed as a major vectors threat different diseases in human being in the world due to its proliferation ability [7]. In the last few decades, the incidence of diseases spread by *Ae. Aegypti* and *Ae. albopictus* increased all around the world. In 1969, the epidemic of dengue was present in 9 countries, but now it has spread to more than 100 countries. The prevalence of dengue has increased expressively, and about half the world population is at risk of contracting dengue viruses from *Aedes aegypti* mosquito [8]. There are 4 types of dengue viruses DNV_1, DNV_2, DNV_3 and DNV_4, the DENV-1 serotype predominated, followed by DENV-2, *Aedes aegypti* is a major vector of dengue and highly threatened in urban areas and *Ae albopictus* is a secondary role and highly threatened in rural areas in Myanmar [9]. The incidence of dengue has grown dramatically around the world in recent decades, with cases reported to WHO increasing from 505430 cases in 2000 to 5.2 million in 2019. A vast majority of cases are asymptomatic or mild and self-managed, and hence, the actual numbers of dengue cases are under-reported. Many cases are also misdiagnosed as other febrile illness [1]. The highest number of dengue cases was reported in 2023, affecting over 80 countries in all regions of WHO. Since the beginning of 2023, ongoing transmission, combined with an unexpected spike in dengue cases, resulted in a historic high of over 6.5 million cases and more than 7300 dengue-related deaths reported [2]. Climate change leading to increasing temperatures and high rainfall and humidity, fragile health systems in the midst of the COVIT-19 pandemic; and political and financial instabilities in countries facing complex humanitarian crises and high population movements [10]. World Health Organization revealed that prevalence of dengue estimates that 3.9 million people are at risk of infection with dengue viruses. The disease is now endemic in more than 100 countries in the WHO Regions of Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific. The Americas, Southeast Asia, and Western Pacific regions are the most seriously affected, with Asia representing around 70% of the global disease burden. The WHO Region of the Americas reported 4.5 million cases, with 2300 deaths. A high number of cases were reported in Asia: Bangladesh (321000), Malaysia (111400), Thailand 150000, and Viet Nam (369000) cases in 2023 [10].

Dengue was first reported in Yangon in 1964, with the first major outbreak occurring in 1970 and spreading to all other States and Regions. Yangon Region continues to have the highest incidence reported in the country. Over time, dengue cases have spread to more townships, and outbreak frequency has increased. Among the vector borne diseases, dengue is the most substantial in Myanmar. According to the Ministry of Health in Myanmar malaria epidemic is declining, although dengue is increasing in many parts of Myanmar, especially in Yangon and a small number of Filariasis and Encephalitic [11]. There were 16,130 cases of dengue fever reported in 2023 across five regions and states, including Yangon (MOH 2024). Ayeyarwady Region, along with Yangon, Mon, Rakhine, and Nay Pyi Taw, reported a significant number of dengue fever cases in 2023. In the same year, Ayeyarwady region saw 16,130 dengue fever cases, with the highest incidence in Hinthada District, followed by Maubin and Pathein districts [12].

Biological control, particularly using larvivorous fish, was important to mosquito control programmed in the 20th century, particularly in urban and peri-urban areas for immediate use in developed and developing countries [13]. Recognizing the high larvivorous potential of *Gambusia affinis*, this fish species was purposely introduced from its native Texas (Southern USA) to the Hawaii Islands in 1905. In 1921, it was introduced in Spain; then from there in Italy during 1920s and later to 60 other countries [14]. Beginning in 1908, another larvivorous fish, *Poecilia reticulata*, a native of South America, was introduced for malaria control into British India and many other countries [14]. In Myanmar larvivorous fish *Aplocheilus panchas* was successfully control the *Anopheles* larvae in domestic water wells in coastal areas of Mon State and Tanintharyi Region and also control *Aedes* larvae in water storage containers in Pa an Township Kayin State [15-17]. And elephant mosquito *Taxorhynchities splenditus* was successfully controlled the water storage container bred *Aedes* larvae in Yangon Region [18].

Synthetic insecticides like deltamethrin, temephos, acetamiprid, metofluthrin, and cypermethrin have been found to be effective against *Ae. aegypti* [19,20,21,22]. However, resistance in *Ae. aegypti* has also been reported against permethrin, deltamethrin, and temephos [23,24,25]. Chemical insecticides are a danger to non-target organisms

[26,27] causing endocrine and carcinogenic problems in humans [28,29]. N, N'-diethyl-3-methylbenzamide (DEET) is a common mosquito repellent [30]. However, the extensive use of DEET has also resulted in harmful effects like allergic reactions and skin irritation, and is also responsible for causing brain disease—encephalopathy in children [31,32]. Keeping in mind the problems associated with chemical insecticides, synthetic repellents, and diseases spread by *Ae. aegypti*, there is a need to find out the natural biological sources to develop new biological based mosquito control tools as larvivorous fish, Dragonfly nymph, *Bacillus thuringiensis israelensis*, carnivorous aquatic plant as *Utricularia macrorhiza* [33]. Therefore, attempt has been made to control *Aedes* larvae in water storage containers using a native larvivorous fish *Colisa labiosus* in Hinthada Township, Ayeyarwady Region.

2. Materials and Methods

2.1. Study Design

Laboratory and field base experimental study design was performed.

2.2. Study Period

The duration of the study was conducted in study areas from May 2023 to October 2023.

2.3. Study areas

Ywar Thit village, Hinthada township as intervention and Tha-Phan-Pin Ward, Ingapu Township as non-intervention villages were selected to control *Aedes* larvae in water storage.

Figure 1. Map of the study sites

2.4. Ethical consideration

The proposal was accepted by DMR Ethical review committee.

2.5. Study population

Aedes adults, larvae, larvivorous fish and water storage containers.

2.6. Mosquito larvae collection

Before intervention, *Aedes* larvae were collected from water storage containers from Dagon Myothit North Township and colony was maintained in DMR Entomology laboratory for laboratory test.

2.7. Native larvivorous fish collection

Native larvivorous fish were collected from creeks, ponds, water pools and gutters by using Yinthet (Myanmar name) (fish catching sieve) in Hinthada Township. And all collected larvivorous fish were carried by plastic bags with oxygen to laboratory of Medical Entomology Research Division, Department of Medical Research Yangon to test larva consuming rate of fish.

2.8. Larval consuming tests

Before testing length of the fish were measured in inch. Then, in laboratory, Larva consuming rates of one, two and three each of *Colisa labiosus* and *Rasbora daniconius* were tested against 3rd and 4th instar laboratory reared *Aedes* larvae in 1Lit, 3Lits and 5Lits water volumes according to WHO [34].

2.9. Sample size and sampling

50 households each were randomly selected from Ywar Thit village, Hinthada township as intervention and Tha-Phan-Pin Ward, Ingapu Township as non-intervention villages. Before intervention all the water storage containers from both selected villages were examined for *Aedes* larval positivity and number of larvae were recorded by visual method [35]

2.10. Intervention

Before intervention, during intervention, after intervention survey was done in both selected Ywar Thit village and Tha-Phan-Pin Ward.

2.11. Treatment of water storage containers

For intervention, larvivorous fish *Colisa labiosus* 2 fish each were introduced in major water storage containers, and 1, 2 fish were introduced in minor and miscellaneous containers according to the size of containers in intervention village of Ywar Thit village to control *Aedes* larvae [35].

2.12. Pre and post intervention survey

Before-intervention survey was done in July. During intervention second and third survey was done in August and September. After-intervention survey was done in October.

2.13. Data analysis

Data entry and processing were made using Microsoft excel software. Larval indices, Key container (500 and above larvae positive container), Key premises ((3 and above containers positive with *Aedes* larvae/house)), CI= % of larva positive container, HI= % of larva positive house, BI= larvae positive containers/100households, pupae/house, pupae/container, pupae/person and pupae/child were calculated.

3. Results

Figure 2 shows that one inch length of 1, 2, and 3 *Colisa labiosus* consumed maximum number of 3rd and 4th instar *Aedes* larvae, 1553±38 in 1 lit, 2991±25 in 3 liters and 4590±36 in 5 liters water volumes within 24 hours. *Rasbora daniconius* was found mean number of 421.67 *Aedes* 3rd and 4th instar larvae consumed in 1 liter, 763.33 larvae consumed in 3 liters and 1245.33 larvae consumed in 5 liters water volume.

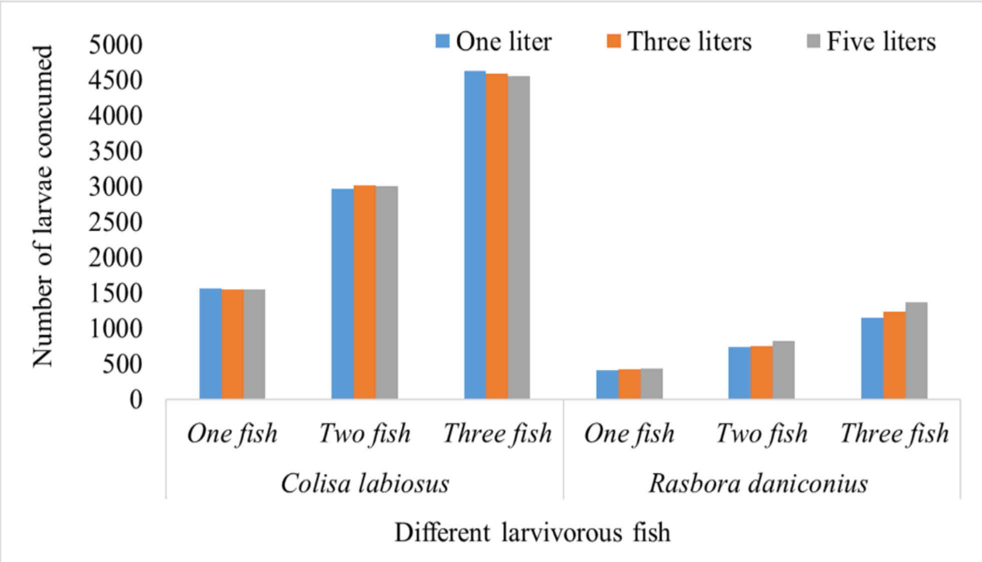


Figure 2. Comparison of larva consuming rate of one inch *of Colisa labiosus* and *Rasbora daniconius* in one, three and five liter of water volumes

Figure 3 shows that containers positivity rate in pre, during and post intervention period using larvivorous fish in test and control villages. After intervention, larva positive containers from Ywar Thit village were found significantly reduced from 64.19% in major, 35.16% in minor and 66.23% in miscellaneous to 0% in major 0.89% in minor and 1.61% in miscellaneous containers. (P<0.05). In non-intervention area of Tha Phan Pin Ward was found larval positivity were increased from 32.37% in major,25.40% in minor and 71.43% in miscellaneous containers to 45.64% in major, 38.96% in minor and 93.62% in miscellaneous containers.

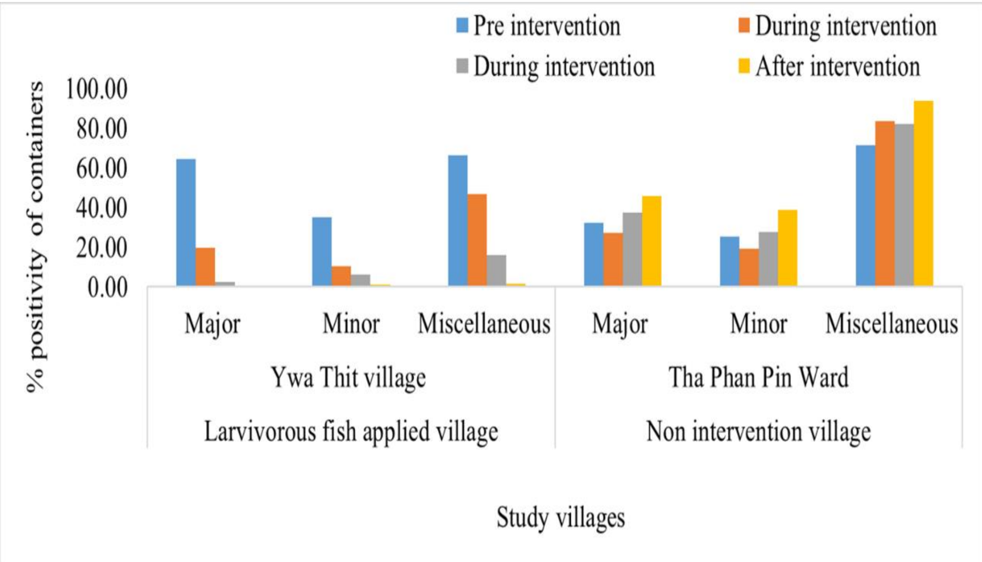
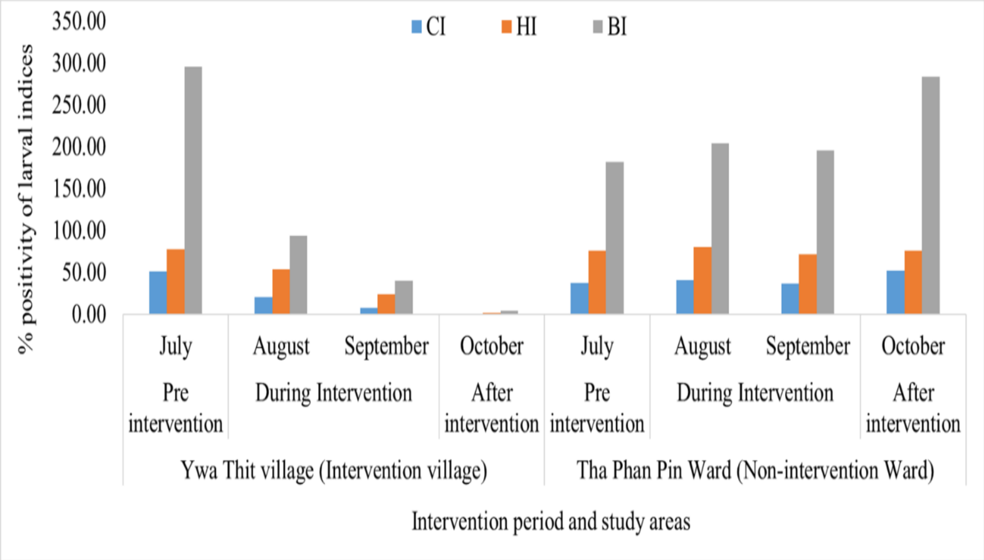


Figure 3. Containers positivity rate in pre, during and post intervention period using larvivorous fish in test and control villages

Figure 4 shows that after intervention by larvivorous fish (*Colisa labiosus*), Larva indices as Container Index (CI), House Index (HI), and Breteau Index (BI) were also reduced from 51.75%, 78% and 296 to 0.79%, 2% and 4 in test area of Ywar Thit village (P<0.05). Although in non-intervention ward (Tha Phan Pin Ward) was found larva indices were increased from 37.3%, 76.00% and 182 to 52.01%, 76% and 284 of CI, HI and BI.

Middle East Journal of Applied Science & Technology (MEJAST)
Volume 8, Issue 3, Pages 82-94, July-September 2025



HI = House index, CI = Container index, BI = Breteau index

Figure 4. Larval indices in Ywa Thit village (intervention) and Tha Phan Pin Ward (non-intervention) villages from Hinthada Township

Table 1. Pupal indices in intervention and non-intervention villages

Pupal Indices	Intervention Area Ywar Thit village Hin Tha da Township		Non-intervention area Tha Phan Pin Ward Ingapu Township	
	Pre-intervention	After-intervention	Pre-intervention	After-intervention
Pupae/house	3.9	0	4.4	74.5
Pupae/container	0.68	0	0.90	13.64
Pupae/person	0.96	0	1.32	22.31
Pupae/child	4.24	0	11.58	196.05

Table 1 shows that Pupal indices in intervention area (Ywar Thit village Hinthada Township) and non-intervention area (Tha Phan Pin Ward, Ingapu Township) and found that in intervention village, Pupal indices as Pupae/house, Pupae/container, Pupae/person and Pupae/Child were significantly reduced from 3.9,0.68,0.96, and 4.24 to 0 each in pupal indices (P<0.05) and in non-intervention ward the pupal indices were increased from 4.4, 0.90, 1.32 and 11.58 to 74.5, 13.64, 22.31 and 196.05 in Pupae/house, Pupae/container, Pupae/person and Pupae/Child in Tha Phan Pin Ward. Pupae/Child was found highest in Tha Phan Pin Ward.

Table 2. Percentage reduction of Key containers and Key premises in before and after intervention period in test and control villages

Study areas/study sites (50 household)	Key Container			Key Premises			Significant difference
	Before Intervention	After Intervention	% Reduction	Before Intervention	After Intervention	% Reduction	
Ywar Thit village (fish) (Test village)	24	0	100% (reduced)	14	0	100% (reduced)	P<0.05 Significance
Tha-Phan-Pin Ward, (Control ward)	16	47	62% (increased)	9	26	34% (increased)	P<0.05 Significance

Table 2 shows that percentage reduction of Key containers and Key premises in Intervention village and percentage increasing of Key containers and Key premises in non-intervention ward in study areas and found that 100% reduction of key containers and 100% reduction of Key premises were observed after introduction of native larvivorous fish *Colica labiosus* in water storage containers of Ywar Thit village Hinthada Township. In non-intervention ward, Key containers and |Key premises were increased from 16 key containers to 47 key containers, that was 62% increased and Key premises was also increased from 9 key premises to 24 key premises, that was 34% increase in Tha-Phan-Pin Ward Ingapu Township Ayeyarwady.

4. Discussion

Mosquito borne diseases continue to be a major problem in almost all tropical and subtropical countries. They are responsible for the transmission of the pathogens causing some of the most life -threatening and debilitating diseases of man, like malaria, yellow fever, dengue fever, chikungunya, filariasis, encephalitis, etc. High density of mosquito borne diseases vectors as malaria vector *Anopheles dirus, An. minimus*, and suspected vectors *An. maculatus, An. culicifacies, An. hyrcanus, An. sundiagus* (etc.), Filarial vector *Cx. quinquefasciatus*, Japanese encephalitic vector as *Cx. tritaeniorhynchus* and suspected vector *Cx. vishnui* and Dengue vectors *Ae. aegypti*, and *Ae. albopictus* were abundantly collected from different areas in Myanmar [3,4,16,36,37]. There are different types of mosquitoes control tools and methods were available in mosquito borne diseases transmitted areas. Chemical methods as insecticides DDT, permethrin, deltamethrin etc., mechanical methods as light traps, carbon dioxide traps, mosquito-net etc., and biological methods as larvivorous fish, larvivorous insects, and plant extracts, essential oils and carnivorous aquatic plant are used for successfully control the mosquito adults and larvae in field condition [18,33,38,39]. *Aphanius dispar* (Ruppell) [18,28], *Aplocheilus panchax*, *Aplocheilus blockii* (Arnold), [19,11] (Common name: Dwarf panchax), *Colisa fasciatus*, and *Gambusia affinis* (Baird & Girard), [18,53] (Common name: Top minnow) are very useable larvivorous fish they can control potentially of vector borne diseases through larval control [38]. Another biological control methods in Laboratory were mentioned that two formulations of *Bacillus thuringiensis var. israelensis* (water dispersible powder and Liquid formulation), were examined for their toxicity against immature and adult stages of *Aedes aegypti* in the laboratory. Bioassay data also showed that pupation percent and adult emergence were affected more by subjecting the larvae to wettable powder than liquid formulation. The mortality values for the adults ranged between 49.33 & 64.23 % when using liquid formulation and wettable powder, respectively [40]. *B. thuringiensis israelensis* was found to be highly pathogenic against mosquitoes. It is a gram-positive, facultative anaerobic bacterium that produces crystal proteins during sporulation that are highly toxic, specific to insect pests, and safe for the environment. These crystal toxins are the optimum alternative to chemical insecticides [41]. Bioassay studies represented that, the first instars are more susceptible to Bt toxin than the fourth instars, also pupae do not affect by the bacteria or its toxin [42]. Native larvivorous fish was found batter that the *B. thuringiensis* due to larvivorous fish destroy or clean all stages of mosquito larvae in water storage containers.

In the present study before intervention, larva consuming rate of native larvivorous fish of Hinthada Township were measured in different water volumes in laboratory and found that one inch length of 1, 2, and 3 *Colisa labiosus* consumed maximum number of 3rd and 4th instar *Aedes* larvae, 1553±38 in 1 lit, 2991±25 in 3 liters and 4590±36 in

5 liters water volumes within 24 hours. *Colisa labiosus* was found higher larva consuming rate than previous study of *Tricogaster tricoptirus* from Kayin State in same water volume. Because one inch of one, two and three *Tricogaster tricoptirus* was consumed maximum number of (687.6, 1566.2 and 1962.6) in 5 liters water volume [43].

In the present study biological control of *Aedes* mosquito larvae in water storage containers using larvivorous fish *Colisa labiosus* in Ywar Thit village and found that a total of 248.75 ±21.8 containers (major, minor and miscellaneous) were examined and found that 32 major, 25 minor and 41 miscellaneous containers were positive for *Aedes* larvae. Although after releasing of larvivorous fish in water storage containers, the *Aedes* larval positivity was significantly decreased from before intervention to after intervention i.e., from 64.19% in major, 35.16% in minor and 66.23% in miscellaneous to 0% in major 0.89% in minor and 1.61% in miscellaneous containers. Although in non-intervention area of Tha Phan Pin Ward was found larval positivity were gradually increased from 32.37% in major, 25.40% in minor and 71.43% in miscellaneous containers to 45.64% in major, 38.96% in minor and 93.62% in miscellaneous containers. Same results were observed in non-intervention villages in other studies by using larvivorous fish *Tricogaster tricoptirus* in Hpa an Township, Kayin State, Elephant mosquito used in Shwepyithar and Dragun fly nymph used in Thaketa Townships in Yangon Region in Myanmar [18,43,44]. Larval indices as HI, CI and BI were also significantly decreased from 51.75%, 78% and 296 to 0.79%, 2% and 4 in Ywar Thit village ($P<0.05$) and Key containers and Key premises were found 100% reduction of *Aedes* larvae after introducing of native larvivorous fish *Colisa labiosus* in water storage containers of Ywar Thit village Hinthada Township. Although in Tha-Phan-Pin Ward (non-intervention ward), Larval indices, as Key containers and |Key premises were increased in Tha Phan Pin Ward. Same reduction of larval positivity was observed in intervention areas and increased in non-intervention area in Thaketa, and Shwepyithar Township in Yangon region [18,44]. Other researcher revealed that biological larvicide for the control of mosquito borne diseases is feasible and effective only when breeding sites are relatively few or are easily identified and treated. Larval control appears to be promising in urban areas, given that the density of humans needing protection is higher than the limited number of breeding sites. Since 1937, fish have been employed for controlling mosquito larvae. Different types of fish have been used so far in this operational technique. However, use of fish of indigenous origin is found to be more appropriate in this operation [38].

Pupal indices as pupae/ house, Pupae/container, Pupae/ person and Pupae/ child were found significantly reduced in test area i.e. 0 each in all pupal indices of Ywar Thit village Hinthada Township and increased in non-intervention Tha Phan Pin Ward. Other researcher also mention that Pupae/child was reduced after introducing *Aplocheilus panchax* in Hpa-an in Kayin State and dragon fly nymphs in Thaketa Township in Yangon Region [18,44].

5. Conclusion

A biological control study using larvivorous fish was conducted in Ywa-Thit village Hinthada Township as intervention village and Tha-Phan-Pin Ward Ingapu Township as non-intervention village in Hinthada District, Ayeyarwady Region. Fifty households each were randomly selected and *Aedes* larvae in water storage containers were detected and calculated by visual method. Before and after interventions, Larval and Pupal Indices, Kye-containers and Key-premises, Pupal indices were measured in both areas. Larva consuming rate of native larvivorous fishes were tested against laboratory reared *Aedes* larvae in laboratory. In Ywa-Thit village, one inch length 2fishes each were put in major and one to two fish each (according to the size of containers) was put in minor and miscellaneous containers for *Aedes* larvae control. Result found that one inch length of *Colisa labiosus* consumed maximum number (1553. ±38) of 3^{rd} and 4^{th} instar larvae within 24 hours. After intervention in Ywa-Thit village, larva positive containers were significantly reduced from 64.19%, 35.16% and 66.23% to 0%, 0.89% and 1.61% respectively in major, Minor and miscellaneous containers. Larval-Indices as CI, HI and BI were reduced from 51.75%, 78% and 296 to 0.76%, 2%, and 4. Key-containers and Key-premises were also reduced from 24 and 14 to 0,0 respectively. Pupal-Index as Pupae/house, Pupae/container, Pupae/person and Pupae/child were also reduced from 3.9,0.68,0.96 and 4.24 to 0 each in Ywa-Thit village (P=0.001). Although after-intervention, in nan-intervention Tha-Phan-Pin Ward, container positivity, larval and pupal indices, Key-containers and Key-premises were found to be increased and Key-containers and Key-premises were found 62% and 34% increased. Native larvivorous fish *Colisa labiosus* is a very effective and suitable control tool for *Aedes* larvae in water storage containers in Hinthada Township. *Colisa labiosus* acts as potential larvivorous effect of *Aedes* larvae and an effective biological control agent, eco-friendly, non-resistance and cost-effective to be considered as potent natural larvicidal agent to control *Aedes* larvae in community. Study suggests that *Aedes* larvae should be maintain continuously and also should be control the *Aedes* larvae in other townships where high density of *Aedes* larvae and dengue cases were available. *Aedes* breeding souses, Key containers and Key premises should be assessed in DHF prone areas.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors made an equal contribution in the Conception and design of the work, Data collection, Drafting the article, and Critical revision of the article. All the authors have read and approved the final copy of the manuscript.

Availability of data and materials

Authors are willing to share data and material on request.

Institutional Review Board Statement

Not applicable for this study.

Ethical Approval

This study was approved by the ethical review committee of the Department of Medical Research, Myanmar.

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