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Bacillus Thuringiensis H-14 with Coconut Water Media: An Alternative Method to Involve Rural Communities in Malaria Control

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Abstract **Background:** Increased community involvement has become a topic in global malaria control and eradication plans. This study aimed to examine a method or system of community involvement in developing Bacillus thuringiensis H-14 with coconut media for malaria control in the Central Bangka District. **Method:** The quasi-experimental research design with qualitative observation method involving 30 participants. Selected participants received training on Bacillus thuringiensis culture in coconut and guidance in assessing the population density of Anopheles larvae in ponds containing mosquito larvae before and after administration of Bacillus thuringiensis. **Results:** The use of local coconuts as a breeding medium for Bacillus thuringiensis H-14 was able to mobilize the community members to participate voluntarily. Participants were able to easily practice Bacillus thuringiensis H-14 culture using local coconut media and determine the location of ponds with Anopheles larvae and assess the population density of larvae in ponds few days after sowing Bacillus thuringiensis H-14. **Conclusion:** The intervention proved successful in mobilizing participants in malaria prevention and control in malaria-endemic areas. The intervention consisted of local organizational structure, education, and utilization of local wisdom as well as encompassing problem identification, planning, and evaluation elements. This method can be applied in other archipelagic areas that are still endemic to malaria.

Keywords Bacillus Thuringiensis, Community Empowerment, Malaria Control, Malaria Vector

1. Introduction

Malaria is an infectious disease and has become a global health problem. The plasmodial species (Plasmodium falciparum) presents a significant health threat to humans [1], [2]. In 2018, the disease caused 405,000 deaths worldwide [3]. From 2010 to 2017, the World Health Organization reported a decrease in malaria incidence by 18%. Despite a significant decline in the last decade, malaria incidence remains high [4]. Approximately 1.8 billion people in Southeast Asia, including Indonesia, are still at risk of contracting malaria; this is more than half the estimated global risk of malaria [5]. Indonesia is one of malaria-endemic countries [6] and up to the end of 2018, there were reports of around 180,000 confirmed malaria cases throughout 26 districts of malaria-endemic areas in the country [7]. Malaria cases occurred in the province of the Bangka Belitung Islands, with 941 cases reported in 2014, which was lower than the 2013 case report (1,834 cases) [8]. The annual parasite incidence (API) in Central Bangka Regency in 2014 was 0.39 per 1000 population, which decreased compared to 2013, which was 2.13 per 1000 population. Although the mortality rate of malaria cases in 2013 and 2014 in all districts was 0%, the Central Bangka Regency was not able to obtain a malaria elimination certificate in 2016 due to the presence of indigenous cases identified from the results of an assessment carried out by the Malaria Elimination Assessment Team of the Ministry of Health in 2015. Indigenous cases are cases that occur due to local transmission [9].

One of the ways to control malaria vectors is by utilizing microbial biological control agents [10]. Bacillus thuringiensis (B.t) is a controlling agent commonly used in malaria vector control since it is not toxic to non-target organisms [11], [12]. Several studies have reported that Bacillus thuringiensis (B.t) can synthesize more than one type of δ-endotoxin. This ability by several strains (such as B.t. alesti, B.t. linear, B.t. kurstaki, B.t. israelensis, and B.t. isolates Salatiga H-14) can be helpful as a natural insecticide [13]–[16]. Bacillus thuringiensis H-14 from the Laboratory of the Center for Disease Vector and Reservoir Research and Development (B2P2VRP) results from the soil habitat isolation in B2P2VRP.

The mass production of Bacillus thuringiensis generally occurs in nutrient yeast salt medium (NYSM), Luria Bertani (LB), and nutrient broth (NB) [17], [18]. B.t. grows and is more harmful to the target when used in cultural media. Bacillus thuringiensis grows best in media with few carbohydrates and amino acids, such as citric acid, glutamate, and aspartate. Bacillus thuringiensis H-14 isolates from Salatiga can grow on coconut water media and effectively control larvae of Aedes aegypti, An. aconitus and Culex quinquefasciatus in laboratory tests. In addition, small-scale field trials (small trials) in the larval breeding pond ecosystem and on sentinel traps in Pabelan Village proved effective in controlling the development of larvae An. Aconitus [19].

Central Bangka Regency is one of the producers of coconuts. However, coconuts are not commonly used by the community as a breeding medium for Bacillus thuringiensis for malaria control due to public ignorance. Thus, it is necessary to transfer the knowledge and skills to the residents of Central Bangka Regency so they can breed B.t. H-14 isolates from Salatiga on coconut media to control malaria vectors in an effort to lower malaria endemicity. Community participation fosters the ability of the community from within the community itself [20]. In implementing this activity, officers or facilitators will help the community recognize their potential, both the potential of natural resources and human resources, so that residents can formulate their problem-solving efforts based on their capabilities.

Public participation in the health sector generally contributes to energy, thoughts or ideas, funds, materials, and other factors. In empowering the community in the health sector, health service providers have a role in working with the community, not for the community. Empowerment aims to form a public health intervention model through community empowerment by controlling malaria vectors in endemic malaria areas. This study aimed to show the process of community empowerment in implementing the use of B.t. H-14 isolates from Salatiga with local wisdom in Central Bangka Regency.

2. Materials and Methods

2.1. Research Sites

The research was carried out in Central Bangka Regency, Bangka Belitung Islands Province, in 2016. Central Bangka Regency consists of 6 sub-districts, namely Pangkalan Baru, Koba, Simpang Katis, Salan River, Namang, and Lubuk Besar. It has a population of 180,903 residents and an area of 2,280.14 km^2. The population density of the regency is 79 people/km^2 with the shape and condition of the land being 4% hilly (395m high), 51% wavy, 20% valley/flat to choppy, and 25% swamp and flat [21].

2.2. Study Design and Data Collection

This operational research aimed to examine a way or system to increase community participation in the development of B.t. H-14 isolates from Salatiga with coconut media. A quasi-experimental design was selected for the study since the study process was a test of the effectiveness of using coconut bacteria to control mosquito larvae in the pond. The study population included all residents living in the study area. The sample size was not calculated using the population proportion formula but was calculated based on the number of residents in the selected villages using the Solvin formula [22]. The calculation used a 95% confidence interval value, with a sample size of 30 participants, while the number of pools selected for the application B.t H-14 local lines bred in coconuts was the number of ponds containing Anopheles sp.

In relation to community empowerment to use larvicides, a participatory approach design was applied in this study. The sample was the population selected to be fostered in breeding B.t. H-14 isolates from Salatiga in coconuts. The sample was determined by purposive sampling on the community to be fostered who lived in the proximity of the mosquito larvae breeding pond.

2.3. Community Empowerment and Implementation of Breeding B.t. H-14

Community empowerment began with workshop activities through training on the introduction and breeding of B.t. H-14. Information on malaria, the life cycle of malaria, ways to eradicate malaria, introduction to B.t. H-14, benefits of B.t. H-14, and breeding methods of B.t. H-14 was provided. In addition, the training on developing B.t. H-14 with the isolation process on coconuts was conducted. The breeding process of B.t. H-14 was optimally carried out within 24 hours. While waiting for the isolation process of B.t. D-14, the team invited participants to find a suitable location of the pond in the village that contained Anopheles sp larvae. Subsequently, the team taught participants to observe the density of larvae by randomly using a dipper with a volume of 350 ml in each pond. After the process of isolating B.t. H-14 was successfully carried out, participants were trained to implement the spread of B.t. H-14 in ponds where there were larvae of Anopheles sp. The spread of B.t. H-14 by participants was based on the expert team's direction using an effective dose of one coconut for a pond area of 1-3 m2. The process was followed by an observation of the density of malaria larvae using a dipper with a volume of 350 ml randomly in each pond and an observation of the density of larvae after 24 hours of sowing coconuts isolated from B.t. H-14. The larval density observations were repeated every 24 hours for five consecutive days.

2.4. Data Analysis

The community empowerment data were analyzed using qualitative observation methods, in which data were collected using the five senses, namely sight, smell, touch, taste, and hearing [23], [24]. This method assesses subjectively in gathering information since it relies on the five senses of the researcher. This study presents the observation results from each stage of the study without involving qualitative measurements. In addition, to determine the successful assessment of community empowerment with regard to environmental aspects, Bacillus thuringiensis H-14 was bred to examine the decrease in larva density in ponds that were breeding places for malaria vector larvae. The residents gained knowledge in observing and calculating Anopheles sp larvae. The decrease in larval density in the ponds can be seen through participants' skills and level of success in conserving and breeding B.t. H-14 in coconuts might be found in the pond.

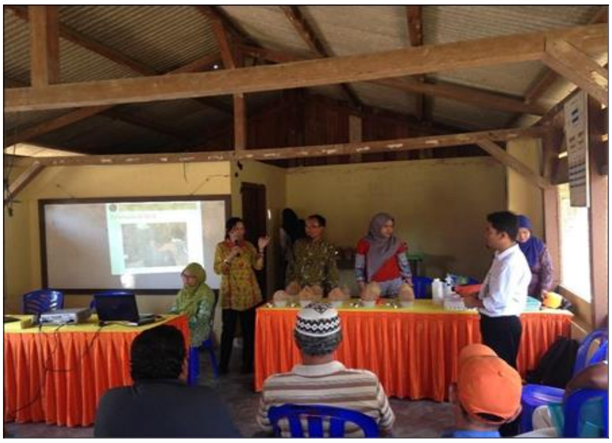
2.5. Ethical Approval and Participation Consent

With letter number LB.02.01/5.2/ KE.007/2016, the National Ethics Commission of the Institute for Health Research and Development of the Indonesian Ministry of Health has granted ethical approval. Prior to data collection, the enumerators obtained participants' written agreement of their participation in the study and the confidentiality of their names and the submitted data.

3. Results

3.1. Recognition and Breeding Training of B.t. H-14

Before participants started breeding B.t. D-14 in coconuts independently, the team first organized a mini-workshop meeting with village residents, village officials and sub-district officials. In addition, those present at the meeting included staff from the Health Office (2 representatives), Puskesmas (2 representatives), neighborhood unit head, researchers and technicians. The meeting was in the form of a debriefing on malaria profiles, malaria vectors and the need for malaria control efforts in the environment using B.t. H-14. During the meeting, the team explained to the attendants how to breed B.t. H-14 in coconuts independently, storage method, larva density data collection, distribution method and evaluation of B.t H-14.



(a)

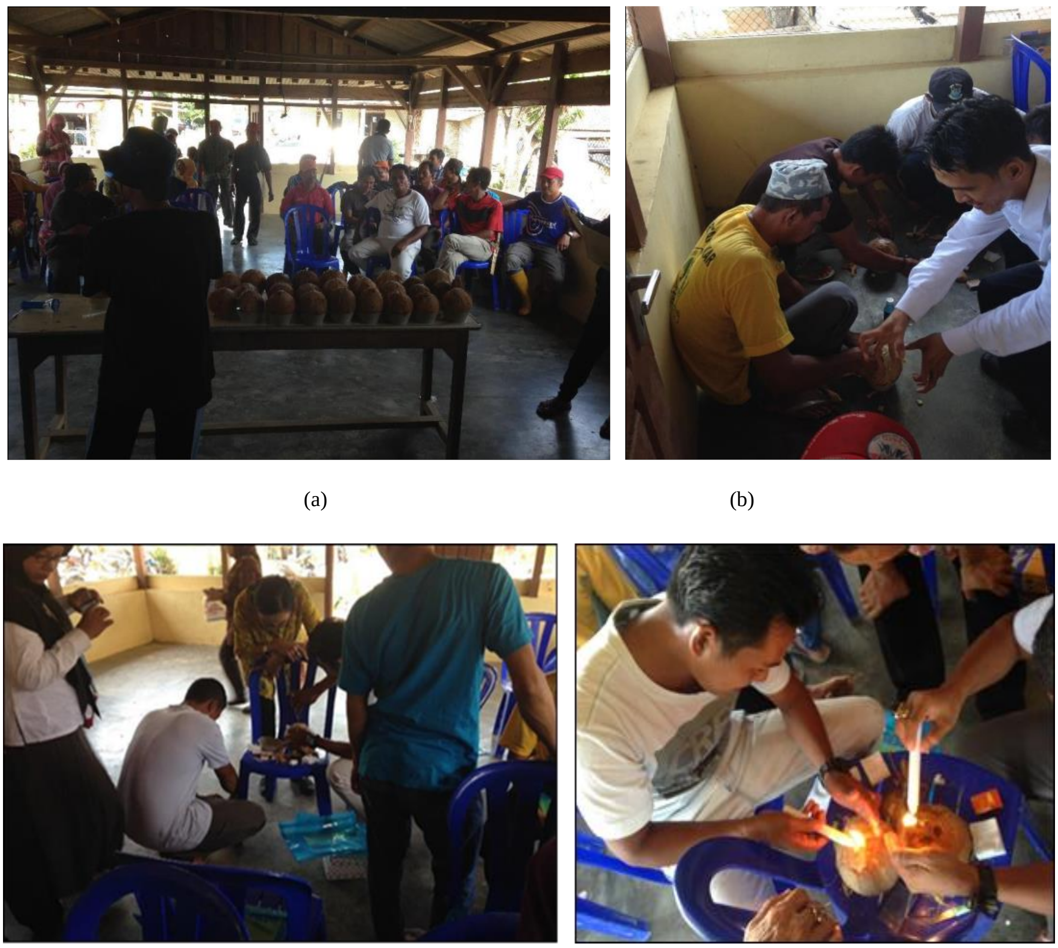
(b)

Figure 1. (a) Training of B.t. H-14 development techniques; (b) Evaluation of training between teachers and participants

In Figure 1, it can be seen that the attendants consisting of participants, community leaders, residents living near the pond and village officials, were enthusiastic in listening to the explanation on the breeding of bacteria B.t. H-14 on coconut media. The research team from the Health Research and Development Agency explained the purpose of the research activity, followed by the empowerment of malaria prevention delivered by representatives from the Provincial Health Office and the Central Bangka Regency Office. At the end of the mini-workshop session, a question-and-answer session related to research activities was conducted. Nearly all participants provided feedback and responded well to the activity. Subsequently, participants received the equipment in the form of knives, candles, alcohol, cotton, pipettes, larvae removal tools and culture B.t. H-14 local strain.

3.2. Development Practice B.t. H-14

After community empowerment and Bacillus bacteria breeding training, participants immediately practiced making bacterial cultures on coconuts using the available equipment (Figure 2). Old coconuts from the village, weighing 400-700 grams, were used as developmental media for B.t. H-14. Participants subsequently cleaned the top part of the coconuts (institutional point) using 70-90% alcohol. After the sterilization process, participants created a hole at the institutional point with a diameter of 1.5 cm. In the next stage, participants entered B.t. H-14 as much as 1-5 ml, and the hole was closed using sterile cotton soaked in alcohol. After the surface of the hole was covered with cotton, participants covered the culture using a drop of wax from a lit candle (Figure 2. b). Participants ensured that the entire surface of the hole covered by cotton was perfect and then coated it with dripping wax. This process is essential for the development of B.t. H-14 and takes place ideally under anaerobic conditions. Coconuts were completely sealed and stored in a room protected from sunlight and at stable room temperature. The incubation process is considered successful if the coconut does not smell rancid and rot.



(a) (b)

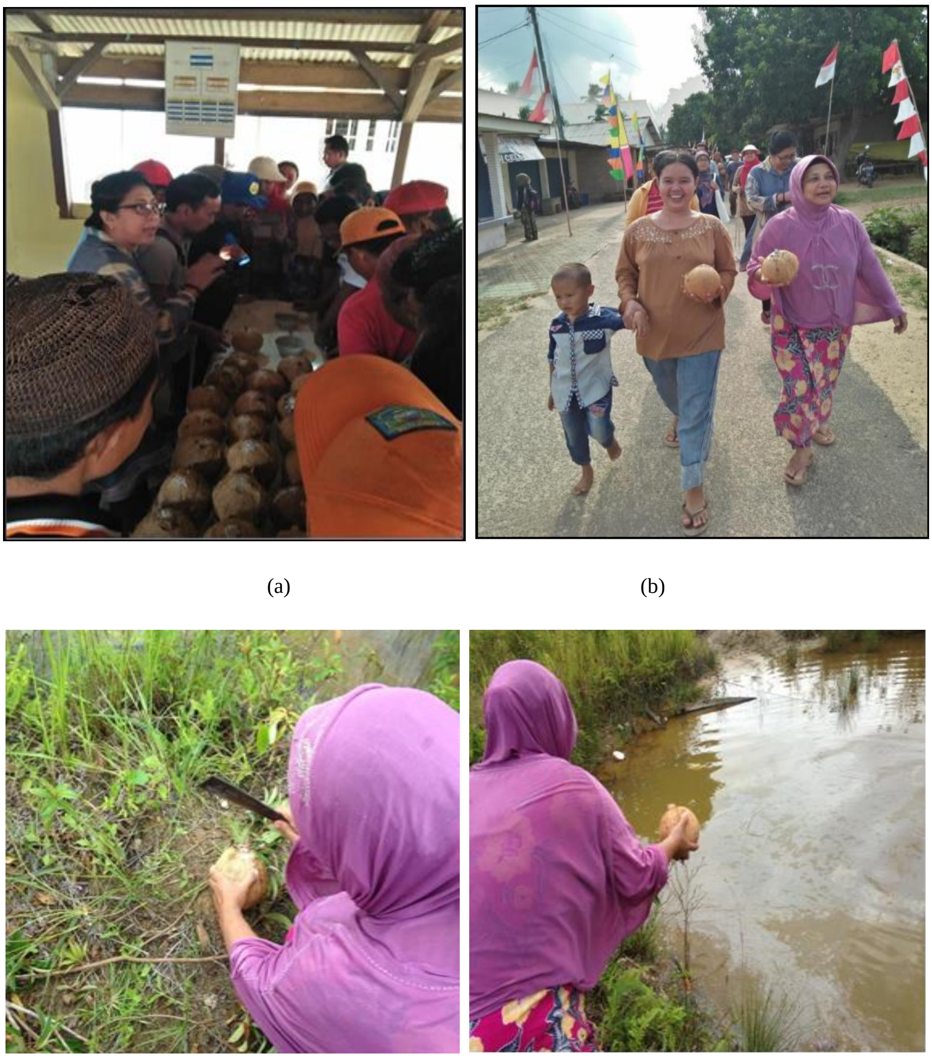
(c) (d)

Figure 2. (a) Explanation of identifying good coconuts for B.t. H-14 breeding process; (b) Creating a hole on the coconuts and cleaning the point of the institution with 70-90% alcohol; (c) Preparing B.t. H-14; (d) Covering the surface of coconuts with wax to enable B.t. H-14 breeding process under anaerobic conditions

3.3. Implementation of B.t. H-14 Stocking

In Figure 3, participants observed the coconuts as the breeding media for B.t. H-14. Participants were trained to identify whether the incubation process of B.t. H-14 on coconuts was successful. During the training, the breeding process of B.t. H-14 was successful. Participants brought coconut media to a pond with Anopheles sp larvae which was inspected the previous day.

The local B.t. H-14 strains, bred in coconuts by the participants, were spread in larval breeding ponds around participants' homes. The amount of spread was adjusted to the size of the pond. The use of one coconut is enough for a pond area of 1-3 m^2. The spread was carried out by opening the cover of the hole and pouring the coconut water into the larval breeding pond. Coconuts that had been empty (without water) were filled with water and then placed in a pond with the coconut hole positioned parallel to the water so that water was able to enter the coconut. Evaluating larvae density was carried out one day and two weeks after application. The evaluation was carried out by participants together with the officers. Environmental parameters such as water pH and temperature were measured before and after applying B.t. H-14 isolates from Salatiga. The following is the documentation of the results of the stocking activities.



(a) (b)

(c) (d)

Figure 3. (a) Coconuts with holes already created; (b) Participants bringing coconuts that had been cultured with B.t. H-14; (c) A participant practicing how to open a coconut; (d) A participant practicing how to spread B.t. H-14 from the coconut

3.4. Malaria Larva Density Observation

The population density of Anopheles sp larvae was observed by randomly sampling using a dipper with a volume of 350 ml in each pond. The larvae obtained were counted and then placed on a plastic baking sheet. Subsequently, participants were trained to observe the type of larvae without using laboratory-scale tools to distinguish between the types of Anopheles sp, Aedes sp, and Culex larvae. Sweeping was carried out 1–2 days prior to the application of the B.t. H-14 local strains in treatment and control ponds to calculate larva density, then one day and two weeks after application of B.t. H-14 local line in coconuts to observe the population density of Anopheles sp larvae (Figures 4a and 4b). The skills and level of success of participants in conserving and breeding B.t. H-14 in coconuts can be seen in the decrease in larval density in the ponds where the stockings were situated.

(a)

(b)

Figure 4. (a) Catching larvae in ponds before administering B.t. H-14; (b) Catching larvae in ponds after administration of local strain of B.t. H-14

4. Discussion

This study found that the participants were able to independently and skillfully breed Bacillus thuringiensis H-14 isolates from Salatiga in coconuts and applied the method in larval breeding sites to suppress the population of Anopheles sp larvae in broodstock ponds at the study site. It is substantial evidence that community participation has an opportunity to help control malaria vectors with the larvicidal method by utilizing local wisdom. Several studies have conveyed the importance of community participation in malaria control. Research in Kenya, Rwanda and Uganda reported the importance of community involvement in formulating appropriate actions toward malaria eradication [25], [26], and designing appropriate implementation strategies for new vector control technologies [27], [28]. Other research shows the role of the community in implementing larva control. In Dar es Salaam, Tanzania, the local government has conducted surveillance of malaria vector microorganisms and larvicides, thereby reducing the prevalence of malaria by training community members and providing them with a small salary [29]. In Sri Lanka, farmer field schools in irrigated rice systems have been integrated with a malaria vector control program, leading to increased knowledge and environmentally friendly mosquito control measures [30], [31].

The community participation method described in this paper is similar to the research on dengue vector control, covering collective action, education, and local organizational structures. In Cuba, community work groups of trained volunteers at the ward level involved the community and stakeholders in decision-making, leading to effective dengue vector control [32], [33]. Providing training on using biological control agents (larvicides) to volunteer committees at the community level in Vietnam resulted in the local elimination of dengue [34], [35]. In contrast, this study outlines a strategy for creating a village-level node where animators and committees inform residents about malaria prevention and control while promoting village-wide action. Community-based larval source monitoring and control can be successful with solid operational management and control. However, regular training and evaluation are necessary to track the efficacy and sustainability of such interventions [36].

For several reasons, the strategy outlined can increase the involvement of community in malaria control. The decision to involve unpaid local volunteers (community and village officials) allows for large-scale intervention, although the method is time-consuming (e.g., holding community workshops). The approach also incorporates components linked to community empowerment, such as problem identification, participatory assessment, monitoring, action, priority setting, adult education, and planning [37], [38]. Making problem analysis a theme for community workshops on malaria should be considered, with self-reported malaria cases as the primary evaluation method.

Efforts to encourage sustainability and adoption of intervention programs need support from government at the national and local levels, with health workers, village heads, and communities acting as parties to support activities [39]. Additionally, the main initiatives of the workshops on community empowerment provide energy to local-level discussions about malaria during the annual cycle. A community workshop on malaria and other health issues is sponsored, therefore the project can be self-sufficient and sustainable [40], [41].

According to several studies, it is necessary to complement vector-borne disease management policies simultaneously with actions in other sectors to address the determinants of malaria effectively [42]. Compared to standalone malaria treatments, community empowerment provides several advantages. Enhanced motivation and volunteerism, the use of local knowledge, support systems, feedback loops, and non-financial incentives can improve the effectiveness of participatory malaria prevention strategies [43], [44]. In order to prioritize case detection, diagnosis, early treatment, and the prevention of further transmission, health personnel must receive training to maintain high performance when implementing malaria elimination initiatives [45].

5. Research Limitations

The limitation of this study is that the implementation of malaria vector larvae control using B.t.H-14 isolates from Salatiga is still in the pilot stage and is limited to Central Bangka Regency due to budget constraints. This study only obtained data with variables that were limited to observations without linking it to the risk of malaria cases and the characteristics of the community in the area.

6. Conclusions

In the Central Bangka Regency, a novel intervention for community mobilization in malaria prevention and control involves the use of local volunteers as healthcare practitioners. Study participants were willing to take part and able to apply technological innovations using B.t.H-14 isolates from Salatiga on coconuts, even though the project was still in the pilot stage. The stakeholders (Provincial Health Office and Central Bangka District Health Office) accepted and supported technological innovations using B.t.H-14 isolates from Salatiga on coconuts to implement it in the Central Bangka Regency area as one of the efforts to eradicate malaria. In addition, Central Bangka Regency needs many local natural resources (coconuts) as B.t.H-14 culture media. There is a need for research that measures the success of implementing control of malaria vector larvae using Bacillus thuringiensis H-14 isolates from Salatiga on the incidence of malaria cases in the community with a more significant number of samples and different locations with heterogeneous ecosystems.

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Conflict of Interest Statement

None declared.

Authors' Contributions

Conceptualization: BR, RS, FPS, and RM. Data curation: BR, RS, RGAM, DL, and MALS. For analysis: BR, RS, DL, and MALS. Methodology: BR, RS, FPS, and RM. Visualization: BR, RS, RGAM, DL, and MALS. Writing – original draft: BR, RS, FPS, RM, and RGAM. Writing – review & editing: BR, RS, DL, and MALS. Project administration: BR, RS, FPS, RM, and RGAM. References: BR, RS, DL, and MALS.

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