Application of Renewable Kenaf Cellulose Nanofiber as a Temephos Nanocarrier for Control of Aedes Aegypti (Diptera: Culicidae) Larvae

Hazirah binti Pengiran
Universiti Malaysia Perlis https://orcid.org/0000-0001-8351-9489
Jahangir Kamaldin (jahangirkamaldin@gmail.com)
Universiti Sains Malaysia
Leo Bey Fen
Universiti Malaya
Hamdan Ahmad
Universiti Sains Malaysia

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Abstract

Larviciding is an effective control method in managing mosquito-borne diseases. However, current carrier materials used in larvicides formulation raised environmental concerns due to its non-renewable origin. Herein, our study aimed to evaluate the application of the renewable source kenaf cellulose nanofiber (KCNF) impregnated with temephos for the control of *Aedes aegypti* mosquito larvae. The morphology of KCNF+T was examined using field emission scanning electron microscope (FESEM) and transmission electron microscopy (TEM) while the quantity of temephos impregnated, released, and retained on the fibers upon dispersion in water were determined using high performance liquid chromatography (HPLC). Subsequently, the bioefficacy of the KCNF+T was evaluated against *Ae. aegypti* larvae. The FESEM and TEM micrograph verified the presence of temephos on the KCNF after the impregnation. HPLC analysis showed the amount of temephos impregnated on KCNF was 97 % of the 0.1 mg temephos. Upon dispersion in water, KCNF+T released 53% temephos and the retention of temephos on KCNF+T gradually decreased to 30, 17 and 7 % on the first, third and fifth month, respectively. The exposure of *Ae. aegypti* larvae to KCNF+T at concentration 0.006 to 0.01 mg/L was effective at 17 - 25 folds in killing *Ae. aegypti* larvae compared to temephos without KCNF. Microscopy examination revealed accumulation of the KCNF on the larval appendages. Overall, our study demonstrated that KCNF made from renewable source is an effective nanocarrier of temephos for the control of *Ae. aegypti* mosquito larvae.

Introduction

Mosquito is one of the common vectors that can spread different kinds of viruses and parasites such as dengue, malaria, Zika virus, Chikungunya etc. (WHO 2020). Dengue fever is one of the important communicable diseases transmitted by *Aedes aegypti (Ae. aegypti)* mosquito. To date, dengue fever has become a global burden with an estimation of 390 million infections per year, and the number of cases has been increased tremendously (WHO 2020). Many factors have been identified as the possible root cause for the rapid spread of the dengue virus worldwide, such as urbanization, increase of human travel, climate change, human behavior, and condition of local environment such as the presence of artificial water containers which offer good larval breeding site (Jansen and Beebe 2010).

One of the elements in the integrated mosquito control programs is the suppression of the *Ae. aegypti* mosquito population during the larval stage by larviciding at the breeding habitat before it turns into adult mosquitoes (WHO 2004; US EPA 2019). Killing mosquitoes at the breeding site is considered as a main reduction source as they are concentrated, immobile and accessible during the larval stage which significantly impacts the overall mosquito population. Moreover, through a targeted approach, insecticide application on the mosquito larvae will be more reliable, cost-effective and reduce the pesticide spread to non-target organisms (WHO 2004; Floore 2006; US EPA 2019).

A commercial larvicide product is formulated with a mixture of active ingredient (e.g. insecticide) and inert ingredients (e.g. surfactant, emulsion, and sand granules) that are utilized to enhance the effectiveness of the insecticide against the mosquito larvae (Badawy et al. 2015; NPIC 2011; Schorkopf et al. 2016; US EPA 2019). One of the common larvicide active ingredient is temephos (O,O,O',O'-tetramethyl O,O'-thiodi-p-phenylene bis(phosphorothioate). The organophosphate pesticide has been used globally against *Ae. aegypti* for the prevention and control of dengue transmission (WHO 2006). It has low acute toxicity and low water solubility of 0.04 mg/L at 25°C (WHO 2011). Since mosquito larvae mainly lived underneath the water surface, the use of carrier material in the larvicide formulation is essential for the pesticides that have low solubility in water and improved the release of the active ingredients into environment (Yusoff et al. 2016). Current carrier materials used in larvicides formulation include mineral sand, synthetic polymer, and polyethylene pellet raised environmental concerns due to its non-renewable origin and caused pollution (Yusoff et al. 2016). For example, larvicides impregnated on polyethylene (PE) pellet for controlled release larvicides are non-biodegradable and led to environmental pollution. The material contained persistent organic pollutants such as polychlorinated biphenyl (PCB), polycyclic aromatic hydrocarbons (PAHs), dichlorodiphenyltrichloroethane (DDT), etc. (Kase and Branton 1986; Rios et al. 2007).

Moving towards sustainable development in promoting good health and well-being as adopted by United Nations in 2015, larvicide formulations should be environmental-friendly by fulfilling the criteria of being renewable, biodegradable, and low toxicity to non-target organisms (UN 2015; Schorkopf et al. 2016). Cellulose is one of the biodegradable natural polymers that can be used as part of environmental-friendly larvicide formulations. It is a polysaccharide consisting of a repeating unit of glucose and a primary constituent of the plant cell wall which can be processed further into nanocelllulose, namely cellulose nanocrystal and cellulose nanofiber (French 2017; Tuerxun et al. 2019). Cellulose nanofiber (CNF) is made of plant pulp such as kenaf through mechanical treatment (e.g. high-pressure homogenizer) with size at one dimension of 1 -100 nm (Nechyporchuk et al. 2016). Kenaf (*Hibiscus cannabinus*) is a fiber plant consisting of inner core fiber and outer bast fiber (Abdul Khalil et al. 2010). Kenaf-based CNF (KCNF) has remarkable physical and chemical properties such as biodegradable, high loading capability, high aspect ratio, hydrophilic, and low crystalline value, thus CNF can be considered as a viable alternative for larvicide nanocarrier (Ong et al. 2017).

Nanocellulose has been widely recognized as an environmentally friendly carrier in biomedicine field but research on the use of nanocellulose as a larvicide carrier is still lacking. To date, only one research was found on the use of modified CNF in the microencapsulation of insect repellent against adult mosquitoes (Lin and Dufresne 2014; Kadam et al. 2019). Herein, in the current study, we aim to evaluate KCNF as a nanocarrier for temephos in controlling *Ae. aegypti* mosquito larvae. Whereby the capability of the KCNF to impregnate, release and retain temephos upon dispersion into water were analysed using HPLC. Subsequently, the larvicidal efficacy was evaluated by dispersing KCNF + T in water containing live *Ae. aegypti* larvae. This study highlights the ability and application of KCNF as a renewable and biodegradable larvicide nanocarrier with enhanced larvicidal bioefficacy without the use of organic solvent.

Materials And Methods

Materials

The raw kenaf bast fibre was manufactured by a local company (Dr Rahmatullah Holdings Sdn. Bhd.), sourced from the kenaf plantation at the east coast of Peninsular Malaysia which is part of the government initiative of sustainable renewable cultivation to replace existing tobacco farming since 2010 under the National Kenaf and Tobacco Board, Ministry of Plantation Industries and Commodities (Basri et al. 2014). The utilization of kenaf as a source to produce cellulose nanomaterial by Nanotechnology and Catalysis Research Centre, Universiti Malaya (NANOCAT) has been reported by Tuerxun et al. (2019). The non-modified KCNF suspension 2.24 %w/w obtained from NANOCAT was utilized as the starting material in this study. It was freeze-dried to produce KCNF in the dry form before impregnating with the technical grade temephos 93% provided by Hextar R&D International Sdn. Bhd. An amount of 5 mg KCNF was impregnated with 0.1 mg temephos solution dissolved in acetone. The impregnation adopted physical adsorption and solvent evaporation technique to produce KCNF with temephos (KCNF + T). The analytical chemistry using high-performance liquid chromatography (HPLC) utilized the standard temephos 98% as reference (CAS No. 3383-96-8, Dr. EhrenstorferTM) and n-hexane with ethyl acetate of HPLC grade as the dilution solvent and mobile phase.

Morphology characterization of KCNF + T using FESEM and TEM

The sample of KCNF + T for the morphological characterization using field emission scanning electron microscope (FESEM) and transmission electron microscopy (TEM) was prepared using the technique described by Tuerxun et al. (2019). The surface morphological characterization was evaluated using FEG Quanta 650 FESEM with an accelerating voltage of 30 kV in high vacuum mode. While the structural morphology was determined using TEM model Libra 120 Zeiss with an accelerating voltage of 120 kV in bright field mode.

Determination of temephos amount impregnated on KCNF, release and retention of temephos using the HPLC

Amount of temephos in the KCNF + T within one week after impregnation (sample A), release in the water dispersed with 5 mg of the KCNF + T (sample B), retained in KCNF + T filtered from the water solution dispersed with 5 mg KCNF + T at 1st month (sample C), 3rd month (sample D) and 5th month (sample E) were determined using HPLC.

Sample A for the HPLC analysis was prepared by extracting temephos from 5 mg KCNF + T using hexane-ethyl acetate (1:1) solvent mixture in 100 mL volumetric flask and sonicated for 1 minute. Then the KCNF + T was filtered from the extraction solvent solution with 0.45 µm nylon syringe filter into a 2 ml HPLC vial. While sample B was prepared by dispersing 5 mg of KCNF + T into 100 ml distilled water to achieve the intended amount of temephos equivalent to 1 mg/L which is the recommended operational dose by the World Health Organization for larvicidal application (WHO 2006). The solution was sonicated based on the technique described by OECD (2017) for 45 minutes at 50 % amplitude to remove the nanomaterial aggregation effect due to the freeze-dried prior dispersion. The solution was then filtered using Omnipore PTFE membrane filter 0. 1 µm (MERCK KGaA, Germany) to remove KCNF particles. Temephos in the filtrate was extracted using the liquid-liquid extraction technique (Henry et al. 1971). Whereas for sample C, D, and E, a 50 mg/L KCNF + T in an aqueous solution that has been stored for 1, 3 and 5 months at room temperature was filtered using Omnipore PTFE membrane filter 0. 1 µm (MERCK KGaA, Germany), and the collected KCNF + T was extracted with hexane: ethyl acetate (1:1) solvent mixture in 100 ml volumetric flask to determine the amount of temephos retained on the collected KCNF + T.

The amount of temephos in all of the five samples was analyzed using HPLC with UV-Vis detector SPD-20A (Shimadzu Corporation, Japan), column type Zorbax RX-Sil 4.6 mm ID x 250 mm, 5 µm (Agilent Technologies, USA) and the HPLC detector was adjusted at 254 nm. Injection volume was 10 µl using an automated injector system with a flow rate of 1 ml/min and oven temperature of 30 to 85°C. Isocratic elution system was utilized with a mobile phase of n-Hexane and ethyl acetate at a 90:10 ratio. Calibration curve was obtained from concentration of 0.1 mg/L to 1.2 mg/L with $r^2 = 0.999$. The amount of temephos in the samples was quantified based on the sample peak area compared with the standard peak area as shown by the HPLC chromatogram, multiple with standard temephos weight over sample weight as guided by CIPAC (1985).

Larvicidal bioefficacy of KCNF + T against Ae. aegypti larvae

The bioefficacy against *Ae. aegypti* was evaluated by exposing live larvae to twelve separate test solutions namely distilled water (control), KCNF only (0.5 mg/L), KCNF + T and temephos only (TM) solutions based on the procedure described by WHO (2005). KCNF + T and TM test solutions were prepared in distilled water at five temephos concentrations ranging from 0.002 to 0.01 mg/L. The *Ae. aegypti* larvae of late third to early fourth instar were obtained from Vector Control Research Unit (VCRU), Universiti Sains Malaysia (USM). The larvae were pre-conditioned in the holding cup at laboratory condition with a temperature of 21 ± 2°C and 12-hour day/night photoperiod while the feed was provided regularly. After the pre-conditioned, 25 healthy larvae were transferred using a strainer into a treatment cup, in a total of 20 cups for each test solution. The bioefficacy of the solutions against the mosquito larvae was based on the number of the larvae been intoxicated (mortality) observed at the 24 and 48 hours upon exposure with the test solutions. The larvae were considered intoxicated when found to be motionless, non-responsive when touched, incapable of rising to the water surface, or not showing diving characteristics (WHO 2005). The percentage of larval under mortality was calculated by total larvae mortality dividing against total 25 larvae that were exposed with test solution following the procedure by WHO (2005). The larva surface morphology was also examined under the stereo microscope model Olympus SZX10 with stereo control Olympus DP20.

Data analysis

The dimensional measurements by the TEM, the temephos quantifications by the HPLC and the larval mortality percentages by the WHO bioefficacy were analyzed using descriptive and inferential statistical tools of the IBM SPSS Statistics version 27 software (IBM Corp 2020). The bioefficacy result was accepted for analysis if the control larvae mortality is less than 5%, and if the control larvae mortality is 5–20 %, the result will be corrected based on Abbot's formula before further analysis (WHO 2005). The bioefficacy result have to be rejected if the control larvae pupate more than 10 % (WHO 2005). The normal distribution of the larval mortality percentage was assessed by Shapiro-Wilks test and Levene's test for homogeneity of variances with significance level set at $p > 0.05$. Kruskal-Wallis ANOVA test was carried out to determine the mean difference between KCNF + T and TM efficacy with significance level $p < 0.05$. A

Dunn's post hoc analysis with Bonferroni adjustments was followed to determine pairs of the test concentrations mean differed significantly (Laerd Statistics 2017). Subsequently, the log dosage-Probit mortality regression analysis was performed to estimate the concentration that caused 50% mortality (LC_{50}) if the percentage mortality of the larvae falls between 10–95 % (Finney 1971).

Results And Discussion

Characterization of KCNF + T using FESEM and TEM

The FESEM and TEM images of the surface and structural morphology of KCNF + T are shown in Fig. 1(a) and 1(b), respectively. The FESEM micrograph in Fig. 1(a) showed the KCNF with rough surface and deposition of solid matter (red circles) among the fibers evidenced the impregnation of the temephos, consistent with finding by Badawy et al. (2015). The author reported the rough surface morphology after incorporation of temephos on the chitosan/alginate capsule. Figure 1(b) shows the presence of grey matter clusters representing the temephos impregnated on the KCNFs. The nano dimension of KCNF + T was 8.90 ± 0.32 nm and 150 ± 7.68 nm for width and length, respectively. The KCNF aggregation shown in the TEM image could be due to the freeze-dried and swelling effects during the temephos impregnation process. Despite the aggregation and swelling effects, KCNF + T fibers were still within the nano-size range (Bhandari et al. 2017; De France et al. 2017; Pötzinger et al. 2017).

Determination of temephos amount impregnated on KCNF, release and retention of temephos using the HPLC

The HPLC result of the temephos amount in sample A, B, C, D, and E are presented in Table 1. The HPLC result of sample A showed that 0.097 mg temephos was impregnated on 5 mg KCNFs resulted 97 % of the temephos has been successfully impregnated onto the KCNF. The impregnation produced KCNF + T with 1.94 %w/w temephos. Sample B indicated 0.051 mg (52.3 %) temephos released from KCNF + T after dispersed in water. As for sample C, KCNF can retained 30, 16, and 7 % of temephos for 1, 3, and 5 months respectively, upon dispersion in water. The high surface area and network structure of the KCNF greatly influenced the capability to impregnate the temephos, consistent with studies by Zhao et al. (2015) and Plappert et al. (2019) whereby the high surface area nanocellulose was found to improve drug loading. It is suggested that the temephos could interact with KCNF through the formation of a weak intermolecular hydrogen bond between O-CH3 of temephos and OH group in the nanocellulose (NCBI 2020a, 2020b; Pietri and Clark 2020). The two aromatic rings composed of non-polar C-C and C-H bonds in the temephos molecules caused the larvicide to exhibit hydrophobic properties (NCBI 2020b). This study suggests that the adsorption and precipitation were the main interaction between KCNF and temephos particles, reflecting the affinity of temephos to nanocellulose as described by Lombardo et al. (2018).

The amount of temephos released was 0.051 mg indicated approximately 53 % of the temephos was being released from the KCNF + T upon dispersion in the distilled water. The swelling ability of nanocellulose in the presence of water and adsorption of temephos at the polymeric structure of KCNF may have enhanced its aqueous dispersibility due to its abundance hydroxyl groups in the non-modified surface of KCNF + T while releasing the temephos through dissolution mechanism (Roy et al. 2014; De France et al. 2017). A possible explanation may due to the presence of water through the dispersion process leads to molecular mobility of the loaded larvicide (Rumondor et al. 2009). This was consistent with another study where hydrophilic carriers was reported to cause swelling and release the drug in the presence of water (Bhandari et al. 2017).

On the other hand, the capability of KCNF to retain temephos on its fibers at 30 % and gradually decreased to 17 – 7 % in five months upon dispersion in water (Table 1), showed that KCNF was able to hold temephos longer. The capability of carrier material to retain active ingredient is essential in active ingredients-carrier interaction so that the pest control efficacy is increased for an extended period and reduced the need to reapply the larvicide (Nuruzzaman et al. 2016). Phuanukoonnon et al. (2006) reported that public ignorance to reapply larvicide at regular interval has led to failure in the mosquito control program. Comparing KCNF + T with a commercial formulation of temephos (e.g: Abate® 1SG), the residual efficacy of the product prepared at the same concentration (1 mg/L) was found to be 15 to 26 weeks (Chen and Lee 2006; Lau et al. 2015). Hence, with the ability to retain temephos for up to five months, it is anticipated that KCNF as a temephos carrier could extend the efficacy for a longer period. However, further residual efficacy studies should be carried out to ascertain the effect on mosquito larvae. It was postulated that the network and fibrous structure of KCNF is the contributing factor that enhanced the ability of KCNF to retain temephos upon dispersion in water. The network structure of KCNF is envisaged to retain the larvicide and creates a tortuous diffusion path in the presence of water after dispersion, which translated into prolonged retained of temephos while indirectly release it slowly (Lombardo et al. 2018).

Table 1
The mean amount of temephos impregnated on the powder KCNF + T (sample A), released in the water dispersed with 5 mg of the KCNF + T (sample B), retained in KCNF + T filtered from the water solution of sample B at the 1st month (sample C), 3rd month (sample D) and 5th month (sample E) determined using HPLC.

Sample	Description of source	Amount of temephos (Mean ± SD) in mg	Temephos impregnated (IP)/ retention (RT)/ release (RL) (Mean % ± SD)
A	Powder KCNF + T (1.94 %w/w temephos)	0.097 ± 0.007	IP in the KCNF + T = 97.0 ± 7.0
B	Water dispersed with sample A*	0.051 ± 0.003	RL into the water = 52.5 ± 2.8
C	Filtered from the sample B – 1st month**	0.029 ± 0.002	RT in the KCNF + T = 29.8 ± 1.9
D	Filtered from the sample B – 3rd month**	0.016 ± 0.002	RT in the KCNF + T = 16.8 ± 2.3
E	Filtered from the sample B – 5th month**	0.007 ± 0.001	RT in the KCNF + T = 6.8 ± 0.9
Note:			
* The 5 mg of the sample A was dispersed into 100 ml distilled water to achieve the intended amount of temephos equivalent to 1 mg/L (WHO, 2006).			
** KCNF + T was collected by filtering the solution with the Omnipore PTFE membrane filter 0. 1 µm (MERCK KGaA, Germany).			

Larvicidal bioefficacy of KCNF + T against Ae. aegypti **larva**

The bioefficacy of all the twelve test solutions against the *Ae. aegypti* larvae are shown in Fig. 2. The *Ae. aegypti* larvae in the control solution (distilled water) showed mortality less than 5 %, therefore Abbot's correction was not needed. The KCNF + T test solutions at the temephos concentration of 0.008 and 0.01 mg/L showed mean mortality above 90 % at 24 and 48 hours of exposure, whereas temephos concentration at 0.002, 0.004, and 0.006 mg/L caused less mean mortality of 5, 21, and 68 %, respectively. For the larvae exposed to TM test solution, the mean mortality was below 5 % for all five concentrations for both 24 and 48 hours. The non-parametric test of Kruskal-Wallis ANOVA showed a significant difference in mortality between KCNF + T and TM test solutions, $\chi 2$ (2) = 319.3, $p < 0.05$. The Dunn's pairwise comparison with Bonferroni adjustments showed that lower concentration (below 0.004 mg/L) of KCNF + T exhibited significantly difference in mortality compared with higher KCNF + T test concentrations (0.006 to 0.01 mg/L). Nevertheless, the percentage mortality was not significant difference ($p > 0.05$) between KCNF + T 0.006 mg/L, 0.008 mg/L, and 0.01 mg/L.

The good bioefficacy shown by the current study is similar to the findings reported by Rahim et al. (2016), with mortality of Aedes mosquito larvae between 35–78 % mortality upon exposure to temephos diluted with ethanol as the carrier solvent at 0.004 to 0.005 mg/L. Whilst, the poor bioefficacy of the TM showed zero to low mortality of *Ae. aegypti* larvae upon exposure to temephos diluted in distilled water without organic solvent due to the poor water solubility of temephos (Rathburn 1985; WHO 2011). The efficacy comparison study between KCNF + T and TM at three test concentrations (0.006, 0.008 and 0.01 mg/L) suggested the use of KCNF as a temephos carrier has notably increased the bioefficacy against the *Ae. aegypti* larvae between 17 to 25 folds. Thus, our study showed that KCNF + T able to cause larval mortality without the presence of hazardous organic solvents.

Table 2 showed the Probit analysis of the larvicidal activity of KCNF + T treatment solutions. The calculated LC_{50} for KCNF + T treatment solutions was 0.005 mg/L for 24 and 48 hours, while LC_{95} was 0.009 and 0.008 mg/L for 24 and 48 hours, respectively. Probit analysis for TM test solution was not carried out due to the percentage mortality did not achieve 10–95 %. The result suggests that KCNF has an influential role as a temephos carrier in controlling *Ae. aegypti* mosquito larvae. The LC_{50} of KCNF + T was found lower with the LC_{50} of the Abate® 1SG, a commercial temephos larvicide product, which is 0.00957 mg/L as reported by Aribudi et al. (2012). Based on the LC_{50} value, it is shown that KCNF as a larvicide carrier able to enhance the larvicidal activity of the temephos even at low concentration (0.005 mg/L).

Table 2
Probit dose-mortality analysis of *Ae. aegypti* larvae exposed to kenaf cellulose nanofiber impregnated with temephos (KCNF + T) and temephos only (TM) at the 24 and 48 hours.

Test solution	*n*	24 h					48 h				
		LC_{50} (95% CL)	LC_{95} (95% CL)	X^2 (df)	P-values	r^2	LC_{50} (95% CL)	LC_{95} (95% CL)	X^2 (df)	P-values	r^2
KCNF + T	2500	0.005 (0.005–0.006)	0.009 (0.007–0.012)	11.928 (2)	0.003	0.949	0.005 (0.005–0.006)	0.008 (0.007–0.010)	6.223 (2)	0.045	0.930
TM	2500	Not analyzed because the mortality did not achieve 10 to 95 %									

The microscopic examination of the larva after 48 hours is shown in Fig. 3. There was no notable morphological change on the larvae exposed in both test solutions. However, the white matter was seen at the thorax, abdomen, and siphon of the larvae exposed in KCNF + T at 0.01 mg/L. It is suggested to be the KCNF because the white matter was not observed for the larva exposed to TM treatment solutions at the same concentration (Fig. 3b).

The larvicidal activity confirmed the release of the temephos from KCNF, which caused the larvae mortality. The affinity of the KCNF + T to accumulate at the larvae appendages further increased the direct larvae contact with temephos retained by the KCNFs, leading to enhance bioefficacy. It is postulated that the KCNF + T ionic surface and *Ae. aegypti* larvae surface charge caused aggregation of the KCNF adhered to the body surface of the insect, which can be seen from the attachment of KCNF at the larvae mouth, leg, and siphon as indicated by red arrows in Fig. 3 (Howse and Underwood 2000; Stadler et al. 2017). Attachment of the KCNF + T surrounding the larvae body may probably result in transcuticular delivery other than gut delivery since there was no presence of KCNF in the digestive tract of the larvae (Howse and Underwood 2000; Phanse et al. 2015). Possible dipole-dipole interaction caused agglomeration and attachment of the nanomaterial at the insect's body surface, disrupted the protective layer, leading to water loss and the death of the insect (Stadler et al. 2017). This is attributed to the triboelectrification generated by the insect's body surface which attracted the nanoparticles (McGonigle et al. 2002; Stadler et al. 2017). Triboelectrification is an electrostatic charging exchange between two surfaces when brought into contact (Schein et al. 2014).

Conclusion

This study demonstrates the capability to impregnate temephos onto the outer and inner surface of the KCNFs up to 97 % from 0.1 mg temephos solution producing KCNF+T with 1.94 %w/w. The study also showed that 53 % of the impregnated temephos can be immediately released by the KCNFs upon initial dispersion into the aqueous solution while exhibiting residual retention up to 7 % up to five months, reflecting the ability of the KCNF to extend the efficacy for a longer period. Furthermore, 53 % of the released temephos has effectively killed 90 % of the *Ae. aegypti* larvae within 24 hours at the concentration of 0.008 and 0.01 mg/L temephos, which is enhanced by the accumulation of the KCNFs on the larvae appendages. These results suggest the potential in employing KCNF made from renewable source as an effective nanocarrier of temephos for the control of *Ae. aegypti* mosquito larvae, without the use of hazardous organic solvent.

Abbreviations

CNF: Cellulose nanofiber;

FESEM: Field Emission Scanning Electron Microscope;

HPLC: High Performance Liquid Chromatography;

KCNF: Kenaf cellulose nanofiber

KCNF+T: Kenaf cellulose nanofiber impregnated with temephos;

PE: Polyethylene; TEM: Transmission Electron Microscopy;

TEM: Transmission Electron Microscopy;

TM: Temephos solution without KCNF

Declarations

Funding and conflict of interest

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Availability of data and material

All related data are included in the manuscript.

Code availability

Not applicable

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Authors' contributions

Conceptualization: Jahangir Kamaldin, Hazirah Pengiran; Methodology: Jahangir Kamaldin, Hazirah Pengiran; Formal analysis: Hazirah Pengiran, Jahangir Kamaldin; Writing-original draft preparation: Hazirah Pengiran; Writing-review and editing: Leo Bey Fen, Jahangir Kamaldin, Hamdan Ahmad; Funding acquisition: Jahangir Kamaldin; Resources: Leo Bey Fen, Hamdan Ahmad; Supervision: Jahangir Kamaldin, Leo Bey Fen, Hamdan Ahmad.

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Figures

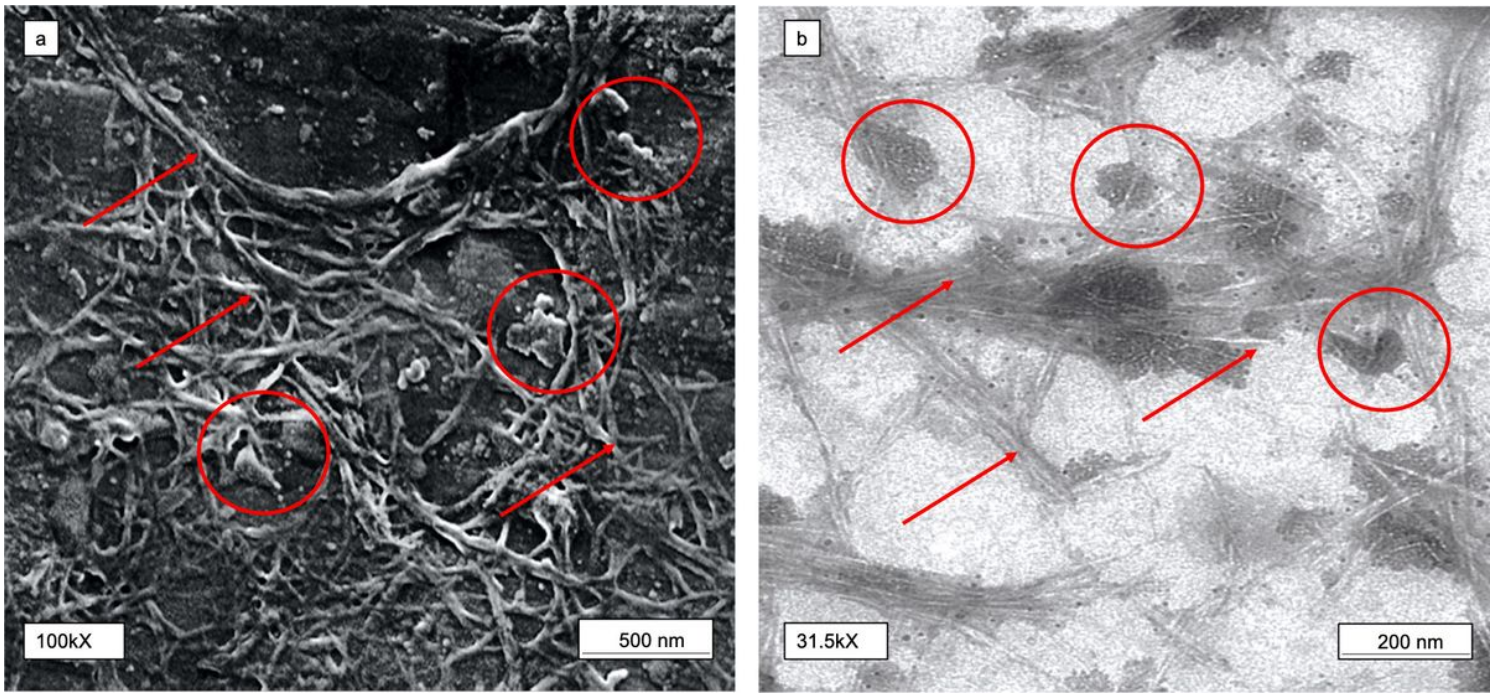

Figure 1

Electron micrographs of kenaf cellulose nanofiber impregnated with temephos (KCNF+T); FESEM micrograph of KCNF+T at 100.0kX (a) and TEM micrograph at 31.5kX (b). Example of the temephos impregnation is indicated by the red circles with the fibers identified with red arrows

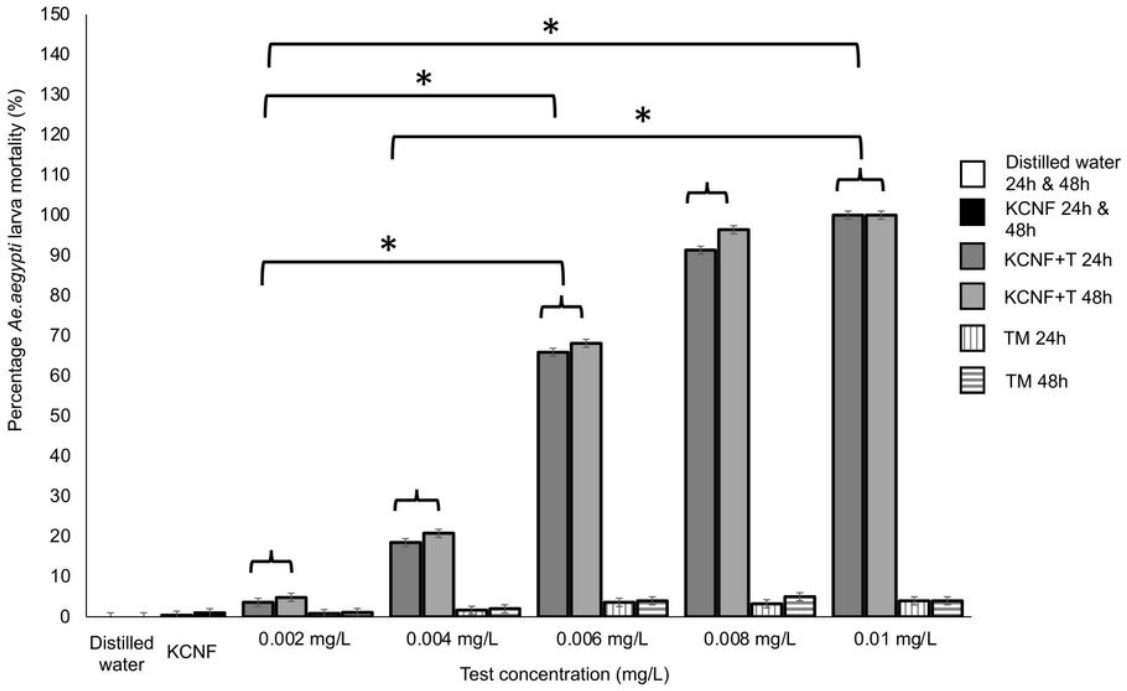

Figure 2

Percentage (%) mortality at 24 and 48 hours of the Ae. aegypti larvae upon exposure with the treatment solutions of the distilled water (control), kenaf cellulose nanofiber (KCNF), temephos impregnated KCNF (KCNF+T) and temephos (TM) only at temephos concentration of 0.002, 0.004, 0.006, 0.008 and 0.01 mg/L. Bars with asterisks (*) indicate a significant difference between KCNF+T test concentrations by Dunn's mean comparison with Bonferroni adjustments, $p < 0.05$.

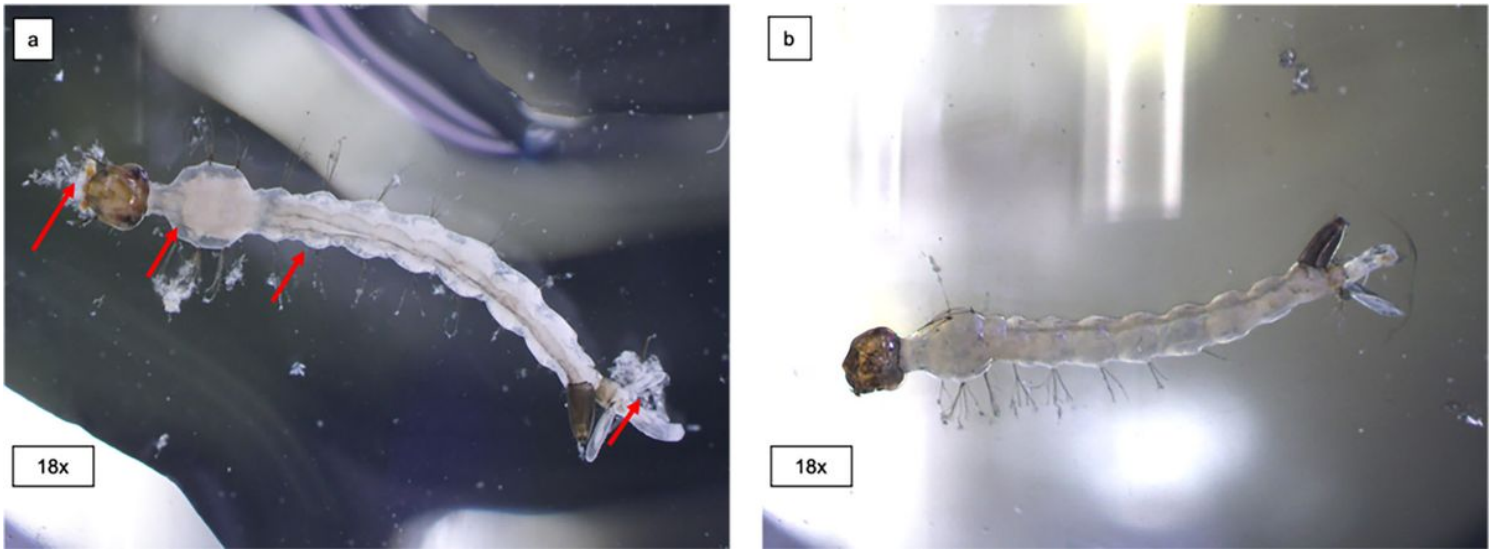

Figure 3

Specimen of Ae. aegypti larva on slide exposed to 0.01 mg/L temephos of the KCNF+T (a), and 0.01 mg/L of TM only solution (b) after 48 hours of exposure at 18x. Red arrow indicated CNF at the thorax, abdomen, and siphon of the larva