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Research Article

DEVELOPMENT OF KARANJ OIL AND CASTOR OIL NANOEMULSIONS AND ITS ENCAPSULATION INTO BEADS FOR CONTROLLED RELEASE LARVICIDAL ACTIVITY

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ABSTRACT

Karanjin and Ricinoleic acid, found in Karanj oil and Castor oil respectively exhibited medicinal and pesticidal properties. Karanj oil and Castor oil has low aqueous solubility, high photo sensitivity which restrict its application for the control of agricultural pests. The present study highlights development of Karanj oil and Castor oil nanoemulsions and its encapsulation into Calcium alginate beads for control release application. The encapsulated beads were characterized by various physicochemical properties such as size, encapsulation efficiency, release kinetics and stability. The beads exhibited a nearly spherical shape and are nonporous in nature. FTIR spectral studies revealed the absence of chemical interactions between active ingredients and crosslinking agent. The encapsulated Karanj oil and Castor oil nanoemulsions showed the potential for controlled release of Karanjin and Ricinoleic acid. The encapsulated beads of Karanj oil and Castor oil nanoemulsions were tested for larvicidal activity and proved to be effective against variety of mosquito species. The advantages of these biopesticidal formulations appear to be an excellent alternative to conventional pesticides.

Keywords: Nanoemulsion, Encapsulation, Control release application, Biopesticides.

1. INTRODUCTION

Essential oils being complex mixtures of volatile organic compounds are generally produced as secondary metabolites in plants. Essential Oils (EOs) are widely used as natural protection agents and environmentally benign pesticides to overcome the pest resistance issue [1-3]. Karanj oil has been recognized in a system of traditional medicines for the treatment of different diseases and ailments of human beings [4]. The crude polar extract from the expelled cake of *Pongamia glabra* containing 20-24% Karanjin exhibited excellent mosquito larvicidal activity against *Culex quinquefasciatus* and *Aedes aegypti* larvae [5]. Castor oil is made by extracting oil from the seeds of the *Ricinus Communis* plant. Castor oil is pale yellow, viscous, non-volatile, non-edible, and non-drying oil. *R. Communis* has various medicinal properties such as anti-inflammetory, diuretic, anticancer, hepatoprotective, antibacterial, insecticidal, hypoglycemic documented by literature [6-10]. The toxic activity of *R. Communis* leaf extract against four mosquito larval species such as *Aedes caspius (Ae. caspius), Culex pipens (Cx. pipiens), Culisetalongiareolata (Cu. longiareolata)* and *Anopheles maculipennis (An. maculipennis)* has been reported [11].

Nanoformulations have the ability of improving biological activity of lipophilic compounds by increasing the surface area per unit of mass [12,13]. Moreover, high kinetic stability, low viscosity and optical transparency make them very attractive systems for many industrial applications [14, 15]. Karanj oil and castor oil nanoemulsion finds applications in fields like agriculture and pharmaceticals. Karanj oil and Neem oil are the two effective botanicals commonly used to control insect pests and mosquito larves [16]. The nanoformulations of single and mixture of non-edible oils with artificial surfactants and natural surfactants are effective for pesticidal activity [17]. Oil extracts from fruits and seeds of Neem (*Azadirachta indica*) and Castor (*Ricinus Communis*), Karanj (*Pongamia glabra*) are explored for their larvicidal activity against vector of filaria, *Cx. quinquefasciatus* and *Aedes agypti* mosquitos [11,18].

The use of encapsulation of active agents for control release applications is a promising alternative to solve the major problem of food or agroproducts [19]. The process of entraping active agents within a carrier material called as encapsulation is the useful tool to improve the delivery of bioactive molecules into pesticides. Controlled release formulations have the potential to reduce the environmental problems associated with the application of pesticides [19]. Microspheres prepared by microencapsulation techniques are the most frequently employed in delivery platforms. Alginates are polysaccharides derived from brown sea weed that are made up of linear chains of a-L-guluronic acid (G) and b-D-mannuronic acid. Alginates are anionic compounds with the ability to produce hydrogels in the presence of divalent cations like Ca^{2+}, which is one of their most important properties [20-22]. Alginate hydrogels are biocompatible polymers having mucoadhesive abilities that have been proven to be useful in a variety of pharmaceutical and biotechnological systems [23,24]. They have been extensively used in the controlled release of pests and drug molecules [25].

The present study focuses on encapsulation of Karanj oil and Castor oil nanoemulsions into calcium alginate beads for control release application which are effective against mosquito larves. In the present study, efficacy of the encapsulated Karanj oil and Castor oil formulations has been studied against various mosquito species. The controlled release technique of encapsulation may reduce the problems associated with microbial and photo-degradation and increase persistence of the bioactive ingredients. It helps to reduce dosage along with application cost and also develop effective, safe and eco-friendly formulations. Alginate based biodegradable polymer is used for encapsulation of nanoformulations. The swelling test, SEM and FTIR analysis of the prepared beads are also carried out.

2. MATERIAL AND METHODS

2.1. Material

Dry fruits of *S. mukorossi* (Soapnut) were purchased from a local shop. Karanj oil and Castor oil were purchased from local Ayurvedic pharmacy. Methanol, Sodium alginate and Calcium chloride were procured from SD Fine Chemicals, Mumbai. Deionized water was used for experimental work.

2.2. Larvae

Third-instar mosquito larvae of *Aedes agypti* and *Culex quinquefasciatus* was provided by Zoonosis department, Haffkine Institute for Training, Research and Testing, Mumbai. The larvae were maintained under suitable temperature and humidity for acclimatisation.

2.3. Methods

2.3.1. Extraction of Sapindus Saponin

Soapnuts were dried at 50°C for 12 h, finely powdered and stored in a dry place. 10 g of Soapnut powder was added into 100 mL methanol (1:10 w/v ratio) and refluxed for 3 h, followed by centrifugation for 10 minutes at the speed of 1000 rpm. The supernatant solution is treated as 10% (w/v) soapnut extract.

2.3.2. Preparation of Nanoemulsion

The emulsions were prepared under the influence of external agitation with the help of magnetic stirrer. Soapnut extract was mixed with the oil phase and then added to the aqueous phase with constant stirring. The resultant mixture was stirred for 2 h with a magnetic stirrer and subsequently ultrasonicated to obtain the nanoemulsion. The prepared emulsion was kept under observation for macroscopic stability for 24 h at 30°C. The emulsion instability was expressed in terms of "creaming," while the milky layer is treated as a stable emulsion.

2.3.3. Encapsulation of stable emulsion

The capsules used in the encapsulation were prepared from mixture of sodium alginate and calcium chloride. Sodium alginate (3% w/v) was prepared in distilled water under constant stirring. 8 mL of prepared nanoemulsion of Karanj oil or Castor oil is mixed with 2 mL of sodium alginate. This resultant solution was then added dropwise into 3% w/v aqueous Calcium chloride solution using 5 mL syringe with constant stirring. The beads of calcium alginate (CA) containing Karanj oil or Castor oil were formed. The beads formed were filtered and washed with distilled water and then dried.

2.3.4. Characterization of encapsulated beads

2.3.4.1. Bead size measurement

To determine the bead diameter, samples of completely dried beads from different formulations were selected, and their size was measured by micrometer screw gauge (Mitutoyo, Japan) with an accuracy of ±0.01 mm.

2.3.4.2. Fourier Transforms Infrared Spectroscopy

A Fourier transforms infrared (FT-IR) spectrometer, Perkins (Liantrisant, UK; serial no 102598), was used to detect chemical interactions between materials. The FT-IR spectra of the beads were then recorded in the wavenumber range of 400-4000 cm^{-1}.

2.3.4.3. Scanning Electron Microscopy (SEM)

Morphology of the freeze-dried beads was examined to study the surface structures of powders by scanning electron microscope (SEM; Leo EVO-40 VPX, Carl Zeiss SMT, Cambridge, UK). The samples were glued onto an adhesive tape mounted on the specimen stub and particles were covered with gold-palladium prior to analysis. Representative SEM images were reported.

2.3.5. Determination of Encapsulation Efficiency

Encapsulation Efficiency (EE) was [26] calculated as the ratio between the oil content in final product to the initial amount of oil to be encapsulated. Oil content in beads was obtained by refluxing a known weight of the beads with 50 mL methanol at 60°C. Methanol was taken because Karanjin and Recenoleic acid are soluble in it. Complete extraction of oil from beads was ensured by refluxing it for 1h. The methanolic extract was diluted with distilled water and then the absorbance of aqueous solution of methanolic extract of Karanj oil and Castor oil was recorded at a wavelength of 217 nm and 214 nm respectively in a UV-visible spectro-photometer (Shimadzu UV-2450, Japan) using distilled water as a blank. The dilution of methanolic extract was done by distilled water. The calibration curves of Karanj oil and Castor oil were obtained by recording the absorption of a known concentration of Karanj oil and Castor oil in distilled water at a wavelength of 217 nm and 214 nm by using UV-visible spectrophotometer. The absorbance values at a wavelength of 217 nm and 214 nm obtained with the respective concentration were recorded and plotted.

2.3.6. Swelling Study of Capsule Beads

The swelling study of the beads was carried out by knowing the percentage of water uptake by the beads at a definite time interval. The dried pre weighed bead samples (W_d) were incubated in distilled water at room temperature. Then the swollen bead samples were removed from the solution at regular time intervals and the weight of the swollen bead (W_s) samples was determined. The experiment was carried out in triplicate and the average value was calculated. The swelling ratio [S.R.(g/g)] was calculated by using Eq.1

$$S.R.\left(\frac{g}{g}\right) = W_s - W_d/W_d \quad (1)$$

Where, W_s = Weight of swollen beads

W_d = Weight of dried beads

2.3.7. Control Release Study

The release profiles of Karanj oil and Castor oil were determined using capsule bead samples (1.25 g) in distilled water (50 mL) with mild magnetic stirring. At a certain interval, 1 mL of the released medium was taken, and an equivalent volume of fresh distilled water was supplemented to maintain a constant volume of the system. Then cumulative release of Karanj oil and Castor oil was measured with UV-visible spectrometer at a wavelength of 217 nm and 214 nm, respectively. All measurements were performed in triplicate and the mean value was used for further data analysis and plotting.

2.3.8. Larvicidal Activity

In the present work, the larvicidal activity of stable formulations was tested using twenty larvae of each mosquito species placed in 250mL sterile beaker containing 200 mL of deionized water at 25±3°C with a photoperiod of 12 h light and 12 h dark. Initially, series of the trial experiments (non-replicated) were conducted with various formulations to optimize the dose with a geometric factor of 2. The concentration of the Karanj oil and Castor oil beads was varied from 0.1 to 2 g (w/v). Each measurement is performed in three replications. The larvicidal effects of the formulations were monitored by recording the mortality after 2, 6, 12, and 24 h of the exposure period.

3. RESULTS AND DISCUSSION

3.1. Preparation of emulsion and encapsulation of stable emulsion

The composition of stable formulations at 30°C is shown in Table 1. The optimised emulsions were successfully incorporated into calcium alginate beads (Table 2).

Table 1: Formulation compositions of Karanj oil and Castor oil emulsions

Formulations (v/v)	Oil (v/v%)	Extract of soapnut(v/v%)	Distilled water (v/v%)	Stability (v/v%)
KE	10	48	42	92(±2)
CE	10	40	50	94(±5)

KE: Karanj oil emulsion; CE: Castor oil emulsion

3.2. Characterization of encapsulated beads

3.2.1. Bead size measurement

The encapsulated beads were measured by micrometer screw gauge. The average bead diameter of Karanj oil emulsion beads and Castor oil emulsion beads was 4±1.0 mm and 5±1.0 mm respectively.

3.2.2. Fourier Transforms Infrared Spectroscopy

FT-IR spectra of pure Karanj oil, Castor oil, Karanj oil emulsion, castor oil emulsion as well as encapsulated Karanj oil emulsion beads and encapsulated Castor oil emulsion beads as two examples of prepared controlled release are shown in Fig.1 and Fig.2.

Table 2: Formulation concentration and characteristics of bead samples

Formulation	KB	CB
Sod- alginate(mL)	2	2
$CaCl_2$(mL)	10	10
Emulsion of Karanj oil / Castor oil(mL)	8	8
Bead diameter(mm)	4±1.0	5±1.0
Encapsulation Efficiency (%)	75±1.0	75±1.0

KB: Encapsulated Karanj oil emulsion beads; CB: Encapsulated Castor oil emulsion beads

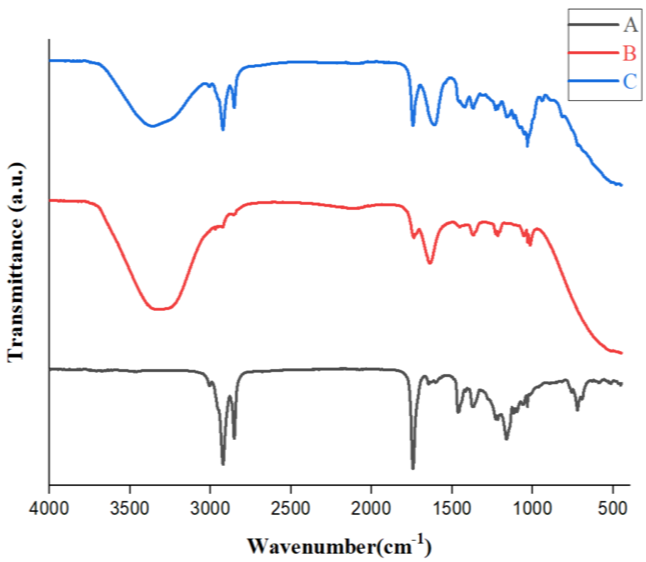

Fig. 1: FTIR spectra of A) Karanj oil, B) Karanj oil emulsion and C) Encapsulated Karanj oil emulsion bead

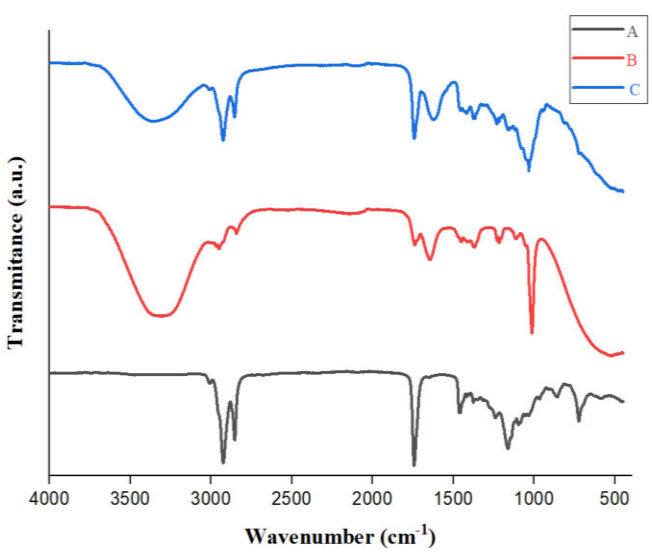

Fig. 2: FTIR spectra of A) Castor oil, B) Castor oil emulsion and C) Encapsulated Castor oil emulsion bead

The FT-IR spectra of karanj oil emulsion and karanj beads show the broad peak at 3357 cm^{-1} due to intermolecular H-bonded O-H stretching. The peaks at 2925 cm^{-1} and 2920 cm^{-1} are assigned as alkyl C-H stretching vibrations. The bands at 1746 cm^{-1} and 1741 cm^{-1} correspond to C=O stretching and C-O stretching of the acetate group. The characteristic absorption bands of alginate are seen around 1431 cm^{-1} and 1648 cm^{-1} wavenumbers which is due to carboxylate stretching vibrations. The bands between 900-1200cm^{-1} are attributed due to stretching absorption of ether groups and -C-O stretching of alcoholic groups. Karanj oil shows C-OH strong band at 1161 cm^{-1} while bands at 1452 cm^{-1} and 1379 cm^{-1} are due to CH_3 bending vibrations.

Castor oil shows C=C stretching at 719 cm^{-1} and C-OH strong bands at 1160 cm^{-1}. The characteristic peaks at 2928 cm^{-1} and 2852 cm^{-1} due to -C-H aldehydic stretchings. Castor oil emulsion and Castor beads show broad bands at 3320 cm^{-1} and 3365 cm^{-1} due to O-H stretching while bands at 1734 cm^{-1} and 1745 cm^{-1} are of C=O stretching of bands. The bands at 1240 cm^{-1} and 1382 cm^{-1} were seen for -C-OH and CH_3 strong stretching vibrations.

The characteristic sharp peaks of pesticides of pure Karanj oil emulsion bead and Castor oil emulsion bead are at 3365 cm^{-1} and 3304 cm^{-1}(O-H stretching), 2929 cm^{-1} and 2925 cm^{-1} (aromatic and/or vinylic C-H), 1745 cm^{-1} and 1748 cm^{-1}(carbonyl stretching), 1032cm^{-1} and 1035 cm^{-1} (C-O groups). These peaks were found with slight shift in place after the formation of KB and CB beads, thereby indicating the absence of chemical interactions between beads and Alginates. This confirms the successful encapsulation of Karanj oil and Castor oil emulsions in bead samples.

3.2.3. SEM images

The beads formed were almost spherical in shape with smooth surface, as shown in Fig.3. The mean particle size was near about 4-5 mm. The external surface of beads containing Karanj oil and Castor oil appeared smoother loaded by calcium alginate beads. Surfaces of Castor oil were more oily than beads containing Karanj oil, may be because of oil release from the cracks surface of beads containing calcium alginate.

3.3. Encapsulation Efficiency

The encapsulation efficiency was calculated as the ratio between the oil content in final product to the initial amount of oil to be encapsulated. From the calibration curves, the concentrations of Karanj oil and Castor oil in bead samples were obtained by knowing the absorbance values. As shown in Table 2, the encapsulation efficiency for all the bead samples is equal to 75% which is a good indication of samples for entrapment of Karanj oil and Castor oil emulsions.

3.4. Swelling Studies

To study the swelling behaviour of bead samples, the dried preweighed bead samples were immersed in distilled water and the weight of the swollen samples was determined at regular time intervals. The swelling behaviour of bead samples as function of time for formulations with Karanj oil and Castor oil beads is shown in Fig.4. The observed trends in the kinetics are similar for all bead samples. Initially, the rate of water uptake increases sharply up to eight hours, followed by a slower rate till the equilibrium is reached.

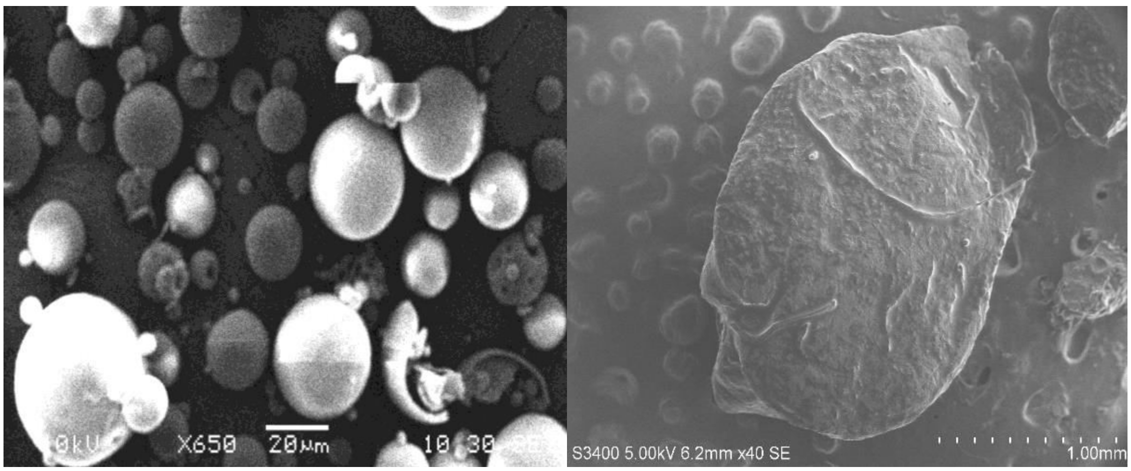

Fig. 3: SEM images of Karanj oil emulsion beads and Castor oil emulsion beads

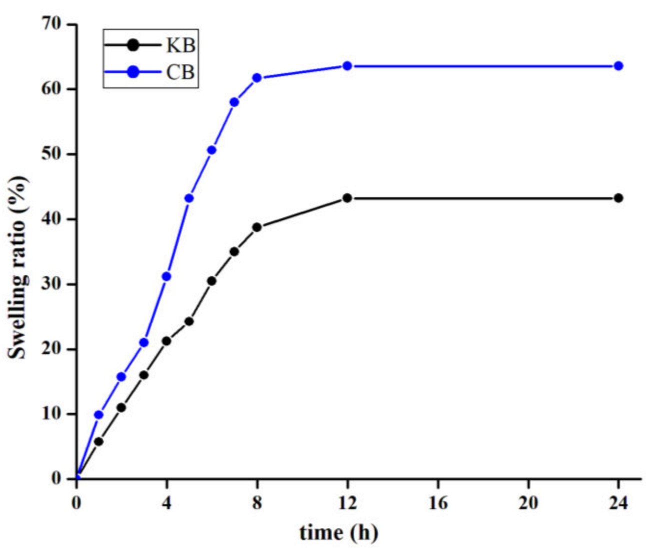

Fig. 4: Swelling behaviour of bead capsules as a function of time for formulations with Encapsulated Karanj oil emulsion beads (KB) and Encapsulated Castor oil emulsion beads (CB)

3.5. Control release studies

The kinetic release was monitored to measure the release rate of the encapsulated oil as a function of the content and the concentration of Karanjin and Ricinoleic acid (Fig. 5). The probable release mechanisms of the oil from microcapsules may include mechanical, chemical, or thermal stimuli as well as diffusion [19, 27].

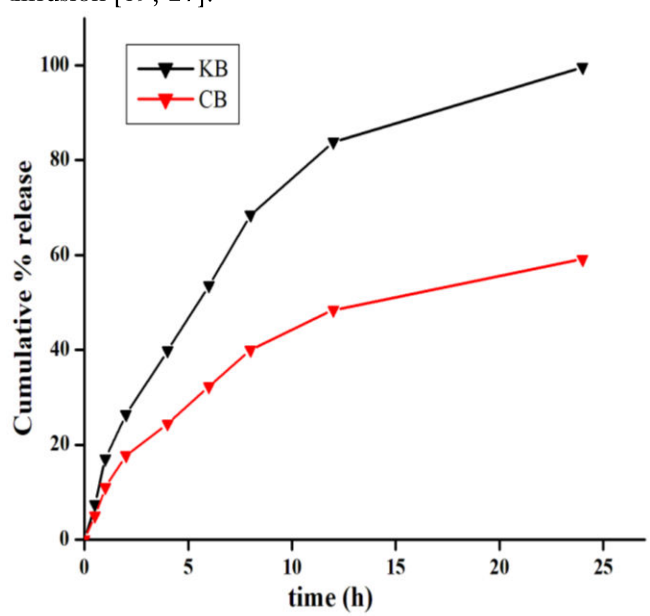

Fig. 5: Release profile of Karanj oil and castor oil from beads of calcium alginate

The cumulative release of Karanj oil and Castor oil from the calcium alginate beads shows initial burst or rapid release attributed to the oils adsorbed on surface and available at the interface while the sustained release was due to embedded oils in bead. Thus, the encapsulation facilitated the controlled release of Karanj oil and Castor oil.

3.6. Larvicidal activity

The results of the larvicidal activity are depicted in Table.3 to Table.6. Different encapsulated formulations of Karanj oil and Castor oil beads evaluated against third instar larvae of Aedes aegypti and Culex quinquefasciatus treated with different concentrations of beads. The mortality rate of Aedes and culex increases with an increase in the concentration of beads. The Karanj oil and Castor oil beads with a maximum 2.7 % (w/v) showed 100 % mortality after 24 h of exposure. The activity of the Karanj oil and castor oil developed, tested after 3 months, was found to be same as that of freshly prepared formulation.

Table 3: Larval mortality data of KB against *Aedes agypti* species

Emulsion Concentrations (w/v%)	**KB** Corrected Larval mortality (%) * of *Aedes agypti*			
	2h	6h	12h	24h
0.3	0(0)	8(1.15)	19(1)	29(1)
0.6	5(0)	8(1.15)	18(1.15)	38(1.15)
0.9	6(1)	10(0)	13(1.15)	39(1)
1.2	5(0)	8(1.15)	16(1)	40(1.63)
1.5	9(1)	15(1.63)	33(2.58)	43(1.15)
1.8	8(1.15)	18(1.15)	34(2.51)	64(2.51)
2.1	11(1)	20(1.63)	26(1.91)	80(1.63)
2.4	18(1.15)	26(1.91)	59(1.91)	88(1.15)
2.7	21(1)	45(1.63)	78(1.15)	100(0)
Untreated control	0.00	0.00	0.00	0.00

**Mean of three replications; Values in parentheses are standard errors. KB: Encapsulated Karanj oil emulsion beads*

Table 4: Larval mortality data of KB against *Culex quinquefasciatus* species

Emulsion Concentrations (w/v%)	**KB** Corrected Larval mortality (%) * of *Culex quinquefasciatus*			
	2h	6h	12h	24h
0.3	0(0)	3(1.15)	6(1)	6(1)
0.6	0(0)	4(1)	8(1.15)	8(1.15)
0.9	3(1.15)	4(1)	8(1.15)	10(1.63)
1.2	4(1)	8(1.15)	9(1)	13(1.15)
1.5	8(1.15)	14(1)	23(1.15)	38(1.15)
1.8	13(1.15)	25(1.63)	40(1.63)	58(1.15)
2.1	16(1.91)	23(2.58)	54(1.91)	68(1.15)
2.4	21(1.91)	41(1.91)	61(1.91)	81(1.91)
2.7	30(1.63)	54(1.91)	88(1.15)	100(0)
Untreated control	0.00	0.00	0.00	0.00

**Mean of three replications; Values in parentheses are standard errors. KB: Encapsulated Karanj oil emulsion beads*

Table 5: Larval mortality data of CB against *Aedes agypti* species

Emulsion Concentrations (w/v%)	**CB** Corrected Larval mortality (%) * of *Aedes agypti*			
	2h	6h	12h	24h
0.3	0(0)	1(1)	5(0)	6(1)
0.6	0(0)	4(1)	5(0)	8(1.15)
0.9	0(0)	4(1)	5(0)	8(1.15)
1.2	1(1)	5(0)	8(1.15)	13(1.15)
1.5	5(0)	11(1)	28(2.58)	43(1.15)
1.8	11(1)	21(2.51)	43(2.58)	55(1.63)
2.1	13(1.15)	15((0)	50(1.63)	75((1.63))
2.4	20(1.63)	36(1.91)	50(2.82)	90(1.63)
2.7	28((1.15)	53(1.15)	88(1.15)	100(0)
Untreated control	0.00	0.00	0.00	0.00

**Mean of three replications; Values in parentheses are standard errors, CB: Encapsulated Castor oil emulsion beads*

Table 6: Larval mortality data of CB against *Culex quinquefasciatus* species

Emulsion Concentrations (w/v%)	**CB** Corrected Larval mortality (%) * of Culexquinquefasciatus			
	2h	6h	12h	24h
0.3	0(0)	3(1.15)	6(1)	8(1.15)
0.6	0(0)	4(1)	8(1.15)	8(1.15)
0.9	3(1.15)	6(1)	9(1)	11(1)
1.2	5(0)	9(1)	15(1.63)	18(1.15)
1.5	9(1)	16(1)	24(1)	26(1.91)
1.8	14(1)	28(1.15)	44(1)	59(1.15)
2.1	18(1.15)	29(1)	59(1)	66(1)
2.4	23(1.15)	41(1.91)	64(1)	83(1.15)
2.7	29(1)	54(2.51)	86(1.91)	100(0)
Untreated control	0.00	0.00	0.00	0.00

**Mean of three replications; Values in parentheses are standard errors, CB: Encapsulated Castor oil emulsion beads*

4. CONCLUSION

Karanj oil and Castor oil nanoemulsion was successfully encapsulated into calcium alginate beads. The results indicated that sodium alginate nanocomposite showed a large encapsulation capacity for Karanj oil and castor oil nanoemulsions. The beads formed were almost spherical in shape with smooth surface. The encapsulated beads of Karanj oil and Castor oil nanoemulsions shows control release up to 24 h. Controlled release of the formulated beads can be more effective in medicine, soil agriculture fields, aquaculture and various other fields. The larvicidal activity of Karanj oil and Castor oil beads are efficacious against *Aedes agypti* and *Culex quinquefasciatus* larvaes. Overall biopesticides of nonedible vegetable oils have opened up new avenues in pesticidal formulations which overcomes the limitations of conventional pesticides.

Conflict of interest

The authors report no conflict of interest.

5. REFERENCES

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