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Encapsulation of Sustainable Nanoemulsion based Insect Repellent against *Bactrocera Species* and Grain Weevils using Throwaways

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Abstract

The repellent effect of the extracts of the *Allium sativum* peel and *Pongamia pinnata* leaves were studied in the present work. Microencapsulation of the nanoemulsion of the extracts was prepared by ultrasonication and tested for insect-repellent activities. Bioassays for the nanoemulsion encapsulated beads were tested in-vitro against the fruit flies (Bactrocera species) and the grain weevils (Sitophilus species). Various properties of the nanoemulsion encapsulated beads such as the morphology of the beads, stability, oil content, and other characteristics were examined. The average diameter of the capsule and other nano characteristics was measured with Scanning Electron Microscope and with an X-ray diffractometer where the crystalline peak exists at around 22º. The shelf life of the encapsulated balls was determined. The encapsulated balls showed evidence of in-vitro repellency against fruit fly and grain weevils at 87% and 92%. The in-vitro toxicity of LC_{50} concentration was shown at 1:3 (L+P) for both grain weevils and the fruit flies when observed at 1hr interval for each of the concentrations after implementation. Thus, the study revealed garlic peel to be a significant insect repellent component being a waste and along with the *Pongamia* leaves has unveiled it to be an appreciable alternate for the synthetic repellents.

Key words: Garlic peel, Grain weevils, Fruit flies, GC-MS, XRD, SEM, LC_{50}, Arena test.

Introduction

Bactrocera species one of the ruinous fruit flies, belonging to the genus *Tephritid* fly resides majorly in the countries of South and South East Asia, Australia and so on. This fruit fly invades cultivated fruits comprising of sugar apple, avocado, banana, citrus fruits, guava, mango, papaya, tomato, etc which breed in the fleshy areas. Feed their larvae and decaying the tissues of the fruits which paves the way for fungi and bacteria to invade which leads to food poisoning. This causes the fruits to rot providing a soaky damp display. As per the survey done in 2012, it had been recorded an annual loss of nearly rupees 2900 crores of mango, guava, citrus crops were outlined in India (1).

Sitophilus species one of the ruinous weevils belonging to the family *Curculionidae,* is an ubiquitous pest present in stored food grains, defiling and damaging the harvested grains causing heavy economic losses to the farmers. These grain weevils invade the cultivated whole wheat grains, whole corns and stored rice,

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where the females' lay eggs, reartheir larvae, altering the microecological state. Over a longer period of time, research on various types of distribution techniques have been obtained for protection against the pests with the usage of plant-based, innocuous extracts (2).

Synthetic pesticides were used but, its ability to extirpate pests has unfavorable effects on human health. Alternates are developed due to its ineffectiveness and high price. The use of synthetic fumigants and the insecticides such as dichlorvos, malathion, methyl bromide, magnesium and aluminium phosphide, chlorpyrifos impose health hazards to humans after consumption and generating toxicity to the environment. Essential oils extracted from the tissues of the plants are often used in the biopesticide formulation which claims to have herbicidal, repellent and insecticidal activities. But they easily deteriorate, vaporize and less hydrophilic in nature. Therefore, eco-friendly and low-cost repellents using the natural source extracts are developed. Garlic, a versatile plant with multiple benefits in which the peels are used in the study of investigating it as a natural repellent for the fruit flies. The main idea of this research is to produce an insect repellent out of waste (3). The peels which are discarded as waste is taken as the main component for this study, because the flies seemed to hover around the fruits and vegetables except for the garlic peel. So, it is found that the peel has an insect repellent property. In order to complement the insect repellency and amplify the effect, leaves from the *Pongama pinnata* tree is also taken as another component for this study. This tree with prevails in most of the streets of India and few other countries of Asia. With its medicinal properties, it can be used for many other ailments. Phytocompounds present in the peel comprising of fatty acid and synthetic auxin, some have demonstrated insecticidal effects and also the *Pongama pinnata* leaves having secondary metabolites (4) with repellent property such as phytol (5) had been identified in the analysis. Therefore, repellents containing the bioactive compounds are developed as an alternate.

For the prolonged use of the insect repellent, nanotechnology comes in action. This helps to get the better of the constraints and enhance the containment of bioactive compounds of both the chemical and biological pesticides. The nanoemulsion carrier used in this study is the sodium alginate, a constituent of brown algae acts as the encapsulating material, where the nanosized molecules will be secured in the matrix of the polymer. The functionality of this technique is to improve the early instability, sustainability, protection, dehydration and so on (6). They are used for various purposes in various industries as emulsifying agent, stabilizer, gelling component and for enhancing the texture. This current study focuses on the nanoemulsion formulation which is synthesized by the process involving high-energy method where oil-in-water (O/W) emulsion comprising of surfactants or emulsifiers with hydrophilic-lipophilic balance (HLB) value of 16.7 which is highly effective for O/W emulsion and a preservative to increase the shelf life and stability. For checking the physiochemical properties of the encapsulated nanoemulsion, various parameters had been performed and observed (7).

Nanoformulated insect repellents decreases the usage of the chemicals, helps in the longer bioavailability of the bioactive substances, retaining the compounds with increases shelf life. The conventional pesticides have proven to be 70% less effective compared to the submicron sized synthesized emulsion because of its repeated overutilization to attain the effectiveness (4). The other formulae of the pesticides such as concentrates, powders and other formats take long term depletion and initiates to pile up in the soil causing adverse effects on health of the living beings (8). The nanoemulsion prepared in the nanospheres with the help of the polymers owing stable properties both physically and chemically with good compatibility and availability is required for augmenting the activity with potency by reducing the toxicity of the insect repellents. These helps

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in diminishing the detrimental consequences on the environment. Other containing the bioactive compounds sustain less optimum quantity of the formulation. But they easily settle on the plant parts (9).

Nanoemulsion being thermodynamically robust consisting of unmixable solvents, where it is widely used in many fields as drug carrier and as other delivery systems. Nanoemulsion is synthesized using the high-energy and low-energy methods and other methods such as the phase inversion method, spontaneous emulsification, high pressure homogenization, ultrasonic emulsification, microfluidization, ultrasonication method and other Brute force methods. Nanoemulsion consisting of submicron particles consisting of optimum emulsifiers and co-surfactants which can be ionic, non-ionic and zwitterionic with an optimum HLB value along with oil, deionised water and a preservative which are subjected to form a monophase. The standard droplet size of the nanoparticles in the emulsion can range upto 500 nanometer. Usually, the nanoemulsion will incorporate non-ionic surfactants because they less likely to get influenced by the factors such as ionic strength, pH and the emulsifier with HLB value of range from 10-16 is considered to be favorable for o/w emulsion. This optimum value can be achieved by combining two surfactants or either with single emulsifier 9-170 nm (8).

Non-ionic surfactants like Polysorbate 20 of size ranging from 99-170 nm (10) can be mixed with the organic phase and aqueous phase by undergoing ultrasonication. For determining the characteristics of the nanoemulsion, photon correlation spectroscopy is used to determine the particle size and polydispersity index, zeta potential where the emulsion is diluted, and the movement of the small droplets of the oil will be measured electrophoretically. Another chacterization method is FTIR which examines the interactivity, cross linking, polymerization and etc., where the functional groups can be assessed (11). Some of the benefits of the emulsion will need quite a low quantity of the emulsifiers with less toxicity for reinforcing the nanospheres or nanocapsules or droplets, has less dissolution for materials with high melting point (6). When the environmental risk assessment has been done, the surfactants are reported to be slightly toxic to the environment due to some modifications in the formulations.

Carrier oil helps in improving the activity of the bioactive substances and reduce the property of evaporation and lowers the degeneracy property which occurs due to isomerisation and oxidation and so on (12). For this organic phase to become a single phase in the nanoformulation, right selection of this oil phase has to be done to facilitate proper generation. The nanoemulsion encapsulated beads are assessed with certain parameters for monitoring its optimum conditions such as the heating and cooling pattern, centrifugation, freeze-thaw pattern and others to check for the phase separation. One of the most followed methods is to check the size of the nanoemulsion is the Dynamic Light Scattering, where the polydispersity index is also measured. The optimum polydispersity index is supposed to be lower than 0.5. Many aspects determine the size of the nanoparticles in emulsion such as the concentration of the surfactant, oil and other constituents like packing. Some previously done researches concluded that with increase in surfactant to decrease in oil can lead to submicron sized droplets. Sometimes the stability of the pesticide is determined by it’s appearance in which the emulsion shouldn’t flocculate, sediment and coalesce, creamy. Temperature plays an important role in determining the stable condition of the formulation, where phase separation will occur. As the number of days increases, a phenomenon named Ostwald ripening occurs (13). In the initial stage of formulation, the particles will be in swift Brownian movements, the particles start to sediment, coalesce and at the end phase separation occurs. This sums up how the properties of the emulsion vary in storage period. Nanoemulsion can be tested on microbes, since they also play a role in decaying plant tissues.

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This nanoemulsion can be less cytotoxic, phtotoxic and genotoxic. The rate of dispersion will be higher compared to the microemulsion. They have this property of releasing controllably and equip the ingredients uptake and the antimicrobial attribute. The smaller the size, more sturdy the nanospheres are and faster the movement of releasing their features (14).

The present work focuses towards extraction and identification of the active compounds from garlic peel and *P.pinnata* leaves using the solvents and to perform *in-vitro* repellent assay and *in-vitro* toxicity assay against the fruit flies and grain weevils. Further a nanoemulsion insect repellent formulation in an encapsulated beads were produced and the end product was characterized for its efficacy and shelf-life studies.

Materials and Methods

Insects and bioassays

For the mass rearing of the grain weevils *Sitophilus spp*, they were supplemented with the powdered mixture of grains such as algae lentils (w/w), wheat flour (w/w) and other cereal grains (5:3:2, w/w), which had started to infest and was screened out maintained at room temperature. *Sitophilus spp* of around 100 of all stages were obtained. *The bioassay was conducted* in invitro conditions at room temperature with Whatman no.1 filter paper and the alginate beads (15).

Sample collection

The periderm of *Allium sativum* L. bulb (Figure 1) and the foliar tissues of *Pongamia pinnata* (L.) Pierre (Figure 2) were carefully separated, thoroughly washed with distilled water to remove adhering impurities, and subsequently shade-dried at ambient temperature. The dried materials were finely pulverized using a mechanical grinder to obtain uniform powder, which was stored in airtight containers for further extraction and phytochemical analysis. The laboratory grade reagents such as ethanol and distilled water were used for the extraction.

Figure 1: Periderm of *Allium sativum* bulb

Figure 2: *Vegetative foliage of Pongamia pinnata*

Extraction

Ten grams of garlic peel powder was subjected to reflux extraction with 200 ml of ethanol as solvent for 3 hours and the residue is filtered, now the filtered residue is used for reflux extraction (figure 3) using 100 ml distilled water. Similarly, ten grams of dried powdered leaves were subjected to reflux extraction with 200 ml ethanol and 100 ml distilled water. The aqueous extracts of peel and leaf were further concentrated by lyophilization at -20ºC (16)

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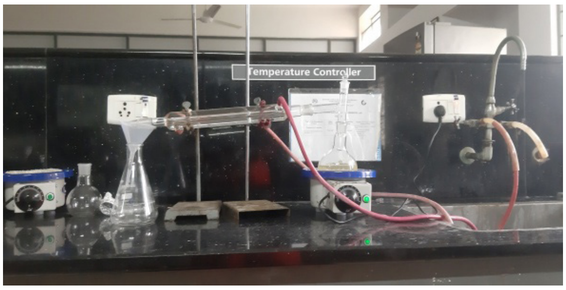

Figure 3: Reflux extraction of *Allium sativum* skin and *Pongamia pinnata* leaves

Phytochemical studies

The various secondary metabolites present in the aqueous and ethanolic leaf and peel extract indicated the presence of certain compounds in the initial phytochemical examination. The phytochemical study was carried out to elucidate the presence of carbohydrates, proteins, alkaloids, phenols, flavanoids, saponin, glycosides, steroids, terpenoids

Identification of compounds

The sample was sent to Indian Institute of Technology, Madras for GCMS analysis. Agilent 8890 GC–MS system was used. The mass data were analyzed. The GC conditions were as follows: column, 30 m x 250 µm x 0.25 µm (Uncalibrated); He carrier gas flow rate, 1.2 mL/min; Run Time: 45.643 min, split ratio, 5:1. The oven temperature was 350 °C for I minute, Average velocity: 40.402cm/sec. The pressure in the column was 11.367 psi (17).

Nanoemulsion formulation

The formulation of oil in water emulsion consisted of 10 various concentrations which had 100 ppm of aqueous peel extract (P), 100 ppm of aqueous leaf extract (L) and the combination of aqueous leaf and peel extracts in the ratio 1:1, 1:3, 1:4, 1:9 and control where it consisted of oil, surfactant and preservative were added. Each of the concentrations were then subjected to magnetic stirring at 350 rpm for 40 minutes and 30-40°C to generate a turbid looking translucent emulsion which was then observed for any phase separation. Later each dose was ultra-sonicated for 20 minutes for 9 cycles with the power of 80 kHz to generate nanoemulsion (18).

Table 1: Quantity of components added in formulation

S.NO	DOSES (ml)	OIL (ml)	SURFACTANT (ml)	PRESERVATIVE (ml)
C1	100 ppm(P)	4	3	3
C2	100 ppm (L)	4	3	3
C3	1:1(P+L)	4	3	3
C4	1:3(P+L)	4	3	3
C5	1:3(L+P)	4	3	3
C6	1:4(P+L)	4	3	3
C7	1:4(L+P)	4	3	3
C8	1:9(P+L)	4	3	3
C9	1:9(L+P)	4	3	3
C10	Control	4	3	3

P = Peel, L = Leaf

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Encapsulaton of nanoemulsion

For the encapsulation procedure, 0.4 g of sodium alginate, a naturally occurring large polymer was mixed in 10ml of citrate buffer. 10 ml of sodium alginate was ultra-sonicated for 30 seconds for 9 cycles at 50 kHz. Successively 1.4 gm of calcium chloride dissolved in 20 ml solution (w/v) is sucked into a 5 ml syringe with a hole of 0.2 cm and released drop by drop into the calcium chloride solution (w/v) to form alginate beads as given in figure 4 of size 0.5 cm.

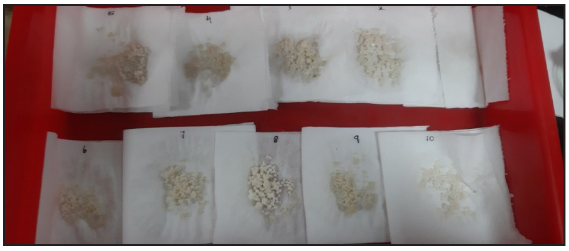


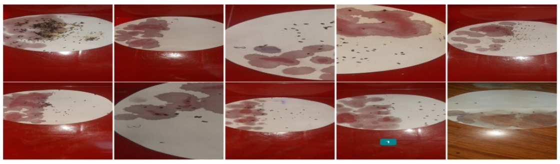
Figure 4: Nanoemulsion encapsulated beads

Thermostability study

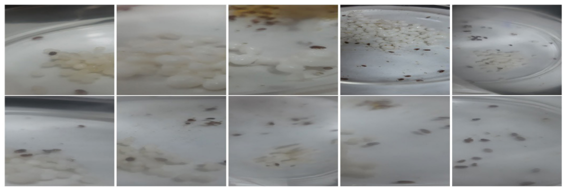
For the stability check of the nanoemulsion , all the doses were exposed to magnetic stirring at 400 rpm for 40 minutes and 30-40°C initially and was observed for phase separation. Later each dose was subjected to heating and cooling cycle alternately for 40 hours with 9 hours interval where the heat temperature was 50°C and the cooling temperature was -20°C as per the (CIPAC) standards.

In -vitro repellent efficacy assay

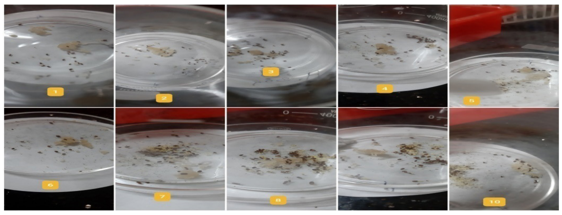
The repellency effect of the extracts of the peel and leaves were tested (19). For the first trial, the repellent action of the liquid formulation was done with 10 different doses, where Whatman filter paper of diameter 13 cm was folded into two equal halves. The filter paper was placed in a petri dish, one for the treatment with various doses and the other half was left untreated. Both the sides of the filter paper were air dried for observing the activity of the emulsions against the fruit flies and weevils of different life stages for 30 minutes. Allowing 6 of the fruit flies and 15 of weevils for each dose to enter, the petri dish was then covered in the first trial which is shown in figure 5(a). For the second trial, the repellent efficacy of 10 different doses was performed using the same filter paper arena test, replacing the liquid formulation with the fresh encapsulated beads which is shown in figure 5(b). Similar number of fruit flies and weevils were allowed to enter as in the first trial. For the third trial, the dried beads were used with the same number of fruit flies and weevils which is shown in figure 5(c).



(a)



(b).



(c).

Figure 5: Three trials of repellent assay

***In-vitro* contact toxicity assay**

To perform this toxicity study (20), 10 petri dishes were used which contained 10 grams of the powdered mixture of grains along with the weevils, where 3 ml of the liquid formulations of various doses were sprayed uniformly on the surface of the mixed grains. These dishes were covered with the inverted 250 ml glass beaker, from preventing the weevils to escape. The percentage of mortality of the weevils were screened, where the number of unviable wee-

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vils were counted along with the total number. According to the method mentioned (21), the mortality percentage can be calculated as,

$$\text{Mortality} = \frac{\textit{(No.of unviable insects)}}{\textit{(Total number of insects)}} * 100$$

Shelf-life studies

After subjecting the encapsulated beads to thermostability studies, the shelf life of the beads was observed. Changes such as losing the moisture, drying, and size shrinkage occurred after 10 days and leaving in the open air at room temperature. When checked for its repellent efficacy, they proved to contain the bioactivity when observed after 30 days. After 30 days, the bioactivity gradually reduces. The shelf life was proven to be 45 days.

Characterization of nano-encapsulated beads

SEM

For the scanning electron microscope (SEM) analysis, the samples were sent to Instrumentation and Analysis department of PSG Institute of Technology, Coimbatore. The images were recorded using Carl ZEISS EVO 18-Germany using the Secondary Electron and Backscattered Electron Modes which is attached with V.4.3 EDS detector with EHT of 5 kV and signal A, SE1.

XRD

For the X-Ray diffractometer (XRD) analysis, the samples were sent to the Instrumentation and Analysis department of PSG Institute of Technology, Coimbatore. The patterns were recorded using the 3rd generation Empyrean, Malvern Panalytical multipurpose diffractometer with MultiCore Optics with X-Ray sources of CuKα (λ=1.54 Å) and Mo Kα (λ=0.71 Å).

Results and Discussion

Sample extraction

After extraction as given in figure 6 from the garlic skin the yield of ethanolic extract was found to be 2.81 g and the aqueous extract was found to be 1.32 g.

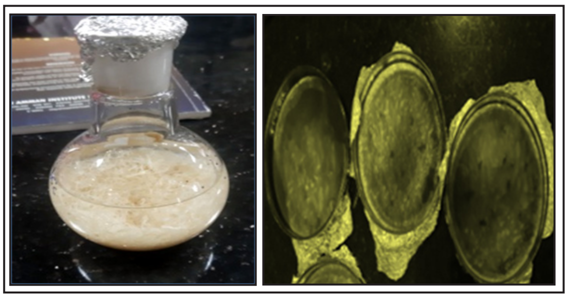

Figure 6: Extraction of bioactive compounds from the garlic skin

After extraction from the leaf as given in figure 7 the yield of ethanolic extract was found to be 2.98 g and the aqueous extract was found to be 1.77 g.

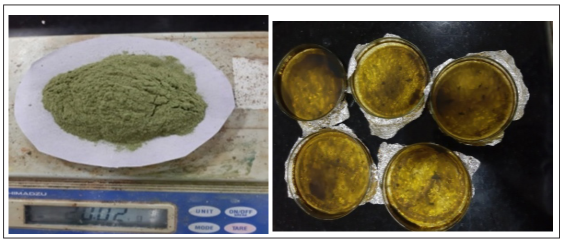

Figure 7: Foliar extract of *Pongamia pinnata* (L.) Pierre

Phytochemical studies of the extracts

The two peel extracts indicated the presence of proteins, flavanoids, saponin and steroids, where the AP has phenols and EP has indicated the presence of glycosides and terpenoids in addition. The two leaf extracts indicated the presence of alkaloids, phenols, carbohydrates and the ethanolic leaf extracts indicated the presence of flavanoids, saponin in addition and the results are given in figure 8.

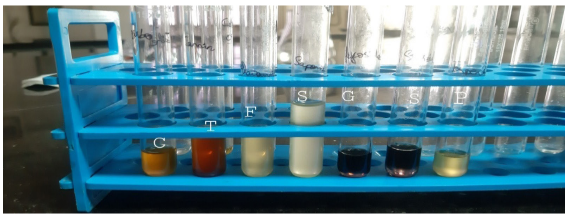

Figure 8: Qualitative phytochemical screening tests performed on the peel and leaf extracts

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Table 2: Results of phytochemical assay of peel extracts

PHYTO CHEMCALS	CARBOHDRA-TES	PROTEINS	ALKLOIDS	TANNINS or PHENOLS	FLAVA-NOIDS	SAPONIN	GLYCO-SIDES	STEROIDS	TER-PENOIDS
AQUEOUS PEEL EXTRACT(AP)	-	+	-	+	+	+	-	+	-
ETHANOLIC PEEL EXTRACT(EP)	-	+	-	-	+	+	+	+	+
AQUEOUS LEAF EXTRACT(AL)	+	-	+	+	-	-	-	-	-
ETHANOLIC LEAF EXTRACT(EL)	+	-	+	+	+	+	-	-	-

+ = presence of the particular phytochemical,
- = absence of the particular phytochemical

GC-MS analysis results

From the results of the repellency test, ethanolic extracts showed high repellency %. A total of 13 compounds were identified in the ethanolic peel extract from the analysis done and the spectral data is given in figure 9. The major compounds are trilinolein, 9-octadecenoic acid (z)-, oxiranylmethyl ester, oleic acid, glycidyl palmitate, fluroxypyr 1- methylheptyl ester and ethyl iso-allocholate which makes the garlic skin to have insect repellent property. Whereas a total of 14 compounds were identified in the ethanolic leaf extract. The common compounds such as Pyrrolizin-1,7-dione-6-carboxylic acid, Propanenitrile,3-[1-[3-(1-pyrrolidinyl) propynyl, 1,2-Nonadecanediol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Heptadecanoic acid, 9-methyl-, methyl ester are present in the leaves of other plant parts. The uncommon compounds in the skin are what make it unique.

Table 4: Compounds in garlic skin extract

PEAK VALUES	COMPOUND NAME	MOLECULAR FORMULA	MOLECULAR WEIGHT(g/mol)
8.159	7-Ethyl-4-decen-6-one	$C_{12}H_{22}O$	182.3
12.150	d-Mannose	$C_6H_{12}O_6$	180.16
15.999	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro4a-phenyl-, ®	$C_{16}H_{18}O$	226.31
22.351	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O2$	284.5
24.979	Oleic Acid	$C_{18}H_{34}O_2$	282.5
26.414	Fluroxypyr 1- methylheptyl ester	$C_{15}H_{21}Cl_2F-N_2O_3$	367.2
27.024	Glycidyl palmitate	$C_{19}H_{36}O_3$	312.5
28.719	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.5
29.287	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)teeth ester	$C_{21}H_{38}O_4$	354.5
29.239	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	$C_{21}H_{38}O_3$	338.5
32.799	15,17,19,21-Hexatriacontatetrayne	$C_{36}H_{58}$	490.8
38.336	Trilinolein	$C_{57}H_{98}O_6$	879.4
45.040	Ethyl iso-allocholate	$C_{27}H_{48}O_5$	452.7

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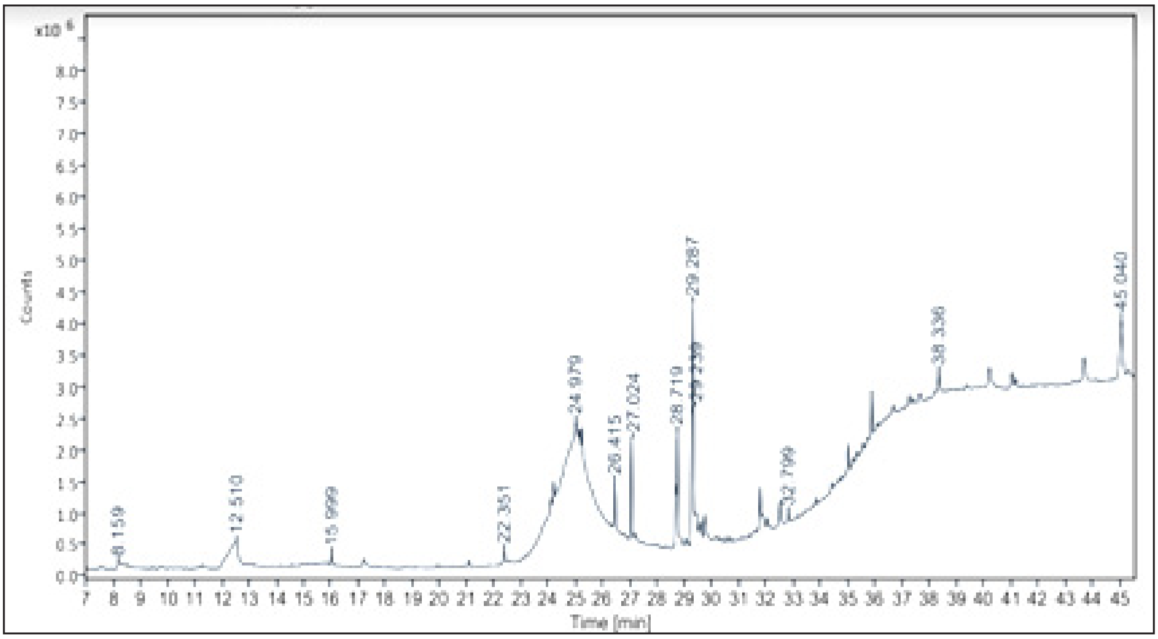

Figure 9: Mass spectral fragmentation pattern of the extract

Repellent efficacy

The liquid nanoformulation, the fresh encapsulated beads with nanoemulsion and the dried encapsulated beads showed high repellent percentage at the concentration C5 for the grain weevils.

Table 5: Average % repellent efficacy of the formulation to grain weevils with 30 minutes for each trial

CONCENTRATON	TRIALS	NO.OF INSECTS	MEAN OF TREATED SIDE(Nt)	MEAN OF UNTREATED SIDE(Nc)	AVERAGE % REPELLENT EFFICACY
C1	1 2 3	15	7	8	13%
C2	1 2 3	15	6	9	33%
C3	1 2 3	15	5	10	50%
C4	1 2 3	15	3	12	75%

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C5	1 2 3	15	1	14	92%
C6	1 2 3	15	4	11	63%
C7	1 2 3	15	2	13	84%
C8	1 2 3	15	2	13	84%
C9	1 2 3	15	3	12	75%
C10	1 2 3	15	7	8	13%

Similarly for the fruit flies, the average repellent efficacy percentage was highest at the concentration C5.

Table 6: Average % repellent efficacy of the formulation to fruit flies with 30 minutes for each trial

CONCENTRATION	TRIALS	NO.OF INSECTS	MEAN OF TREATED SIDE (Nt)	MEAN OF UNTREATED SIDE (Nc)	AVERAGE % REPELLENT EFFICACY
C1	1 2 3	6	3	3	19%
C2	1 2 3	6	3	3	20%
C3	1 2 3	6	4	2	36%

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C4	1 2 3	6	4	2	61%
C5	1 2 3	6	5	1	87%
C6	1 2 3	6	5	1	72%
C7	1 2 3	6	4	2	80%
C8	1 2 3	6	4	2	80%
C9	1 2 3	6	3	3	50%
C10	1 2 3	6	3	3	19%

Toxicity assay results

The Lethal Concentration 50 (LC50) for the grain weevils and fruit flies was found to be 5th concentration, at 1:3 (L+P) and the Lethal Concentration 90 (LC90) for the grain weevils was found to be 9th concentration, 1:9 (L+P) and for fruit flies it was found to be 8th concentration 1:9 (P+L) which is shown in figure 10 and figure 11.

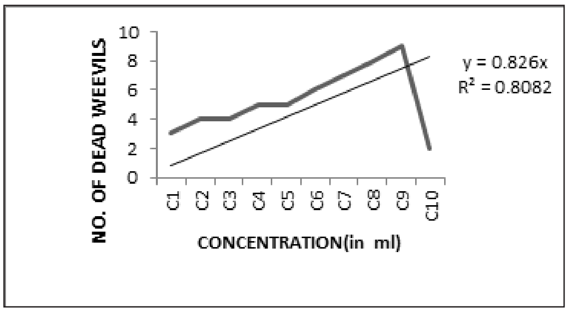

Figure 10: Toxicity assay with weevils

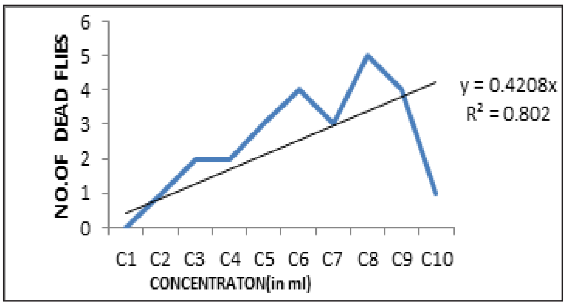

Figure 11: Toxicity assay with fruit flies

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SEM analysis results

The morphology of the surface of the encapsulated beads is shown with magnification of 250x, 500x, 100x, 2.50 kx, 1.00 kx, 5.00 kx, 10.00 kx. Different magnifications show different internal surfaces like rough surfaces, lesions and web of chains of alginate and others. The average size of the beads is 300µm and 200µm with a width of 9.5 mm and 8.5mm is shown in figure 12 (a) and (d) with a magnification of 100x, figure 12 (b) and (c) with the magnification of 500x and 100x shows a substantial change in the surface of the beads. The figure 12 (e) shows the web of alginate network formed inside the beads which is infused with the components of the insect repellent with a magnification of 1.00kx. The small lesions like structure inside the beads is shown in figure 12 (f),(h),(i),(m) with magnifications of 2.50kx, 5.00kx. The more magnified view of the lesion like morphology is shown in figure 12 (g) with the magnification of 1.00kx. The figures 12 (j), (k), (l) shows the rough surfaces and cracked surfaces of the bead.

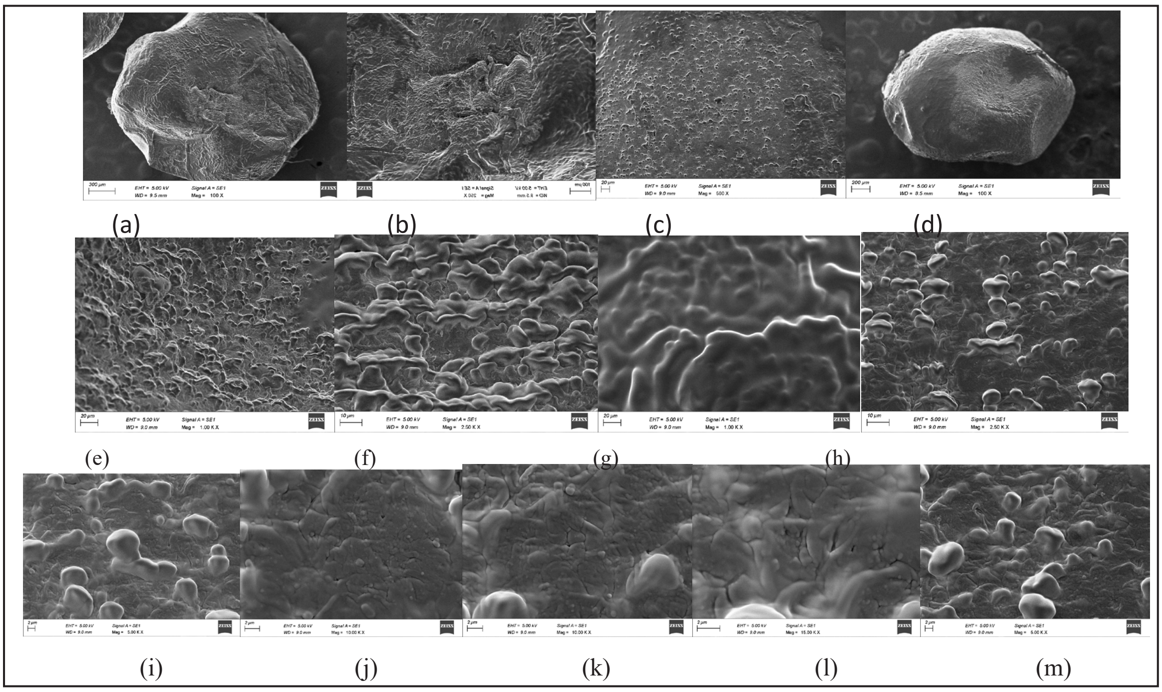

Figure 12: Backscattered Electron Images of the encapsulated beads

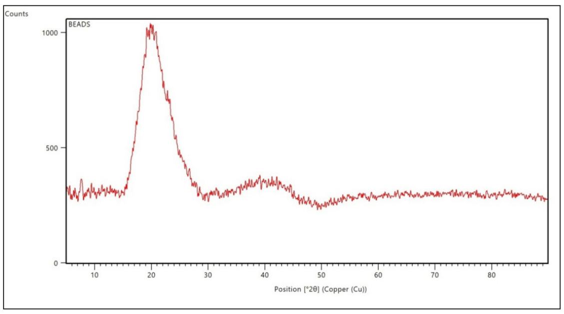

Figure 13: Diffractogram of the nanoemulsion

XRD analysis results

With the XRD pattern generated it is found that amorphous peak exists around diffraction angles of 22º of 2θ in figure 13. Other observations of the crystallites has already been reported.

Discussion

In the study reported, it describes that the oil-in-water emulsion was prepared by the

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high-energy emulsification method ultra-sonication was used to avoid the phase separation process. The extracts such as the aqueous garlic skin extract (5%) and aqueous *P.pinnata* leaf extract (5%) were subjected to encapsulation using the sodium alginate (2%) including oil (3%), emulsifier (2%) and a preservative (2%) by bead formation method. Aqueous extracts of the samples were taken for the formulations since the ethanol extracts were reported to be toxic in the previous work (Adane Adugna Ayalew, 2020). Sometimes the water-soluble herbicides like the sprays or the wetted powders can adulterate the aqueous environment. So, the extracts were taken along with the O/W nanoemulsion, produced by the high-energy method exhibited comparatively low opacity stating that it proceeded the thermostability test very well. When the nanoemulsion was subjected to magnetic stirring, there occurred phase separation and was quite unstable. The nanoemulsions were prepared in ten different concentrations (Table 1) which includes the concentrations of the extracts, oil, surfactant and preservatives. All the ten concentrations were encapsulated into sodium alginate beads. When the nanoemulsion encapsulated beads of ten different concentrations were tested for repellent efficacy, amidst the ten different concentrations the 5th concentration, 1:3(L+P) showed the highest repellent activity for both the grain weevils and fruit flies. Further, the fifth concentration was chosen for SEM analysis and XRD analysis to check the morphology of the encapsulated beads (fig 13) and to check the diffraction patterns of the components in the encapsulated beads to check if it is crystalline or amorphous (fig 14) and it wa amorphous. The morphology of the beads had a coarse surface with lesion-like structure when seen with different magnifications. The average size of the bead was found to be 300 µm with a width of 9.5 mm (22).

The stability of the loaded beads was monitored and it turned out to be stable as interpreted after admitting them to the heating and cooling cycle (23). However, the stability remained till the shelf life of the encapsulated beads. When the characterization of the nanoemulsion was done with magnetic stirring and centrifugation for the selected concentration C5, no phase separation occurred after altering the formulation, though they looked a little turbid. The in-vitro toxicity assay was done for all ten concentrations with the liquid formulation. Initially, all the insects tested were dead because of high concentrations of the components. When the concentrations of the nanoemulsions were altered, the toxicity of the nanoemulsions was reduced. When the LC50 concentration was obtained where half of the population subjected were dead, it was turned out to be C5 (1:3(L+P)) for both fruit flies and rain weevils and the LC90 concentration where 3/4th of the population will be dead turned out to be C9 (1:9(L+P)) for grain weevils and C8 (1:9(P+L)) for fruit flies.

The two compounds in garlic skin where it expressed an appreciable insect repellent activity were Fluroxypyr 1- methyl heptyl ester and oleic acid (fig 10) when GC-MS analysis was done. The presence of phytol in the *Pongamia pinnata* leaves indicated by GC-MS analysis exhibited insect repellent activity. A previous study reported, that when compared with the pesticides or insecticides or herbicides used in agricultural application which is mostly generated as microemulsion, nanoemulsion based herbicides or insect repellents or pesticides exhibited more benefits. Modern time insect repellents or pesticides come with more efficacy and advantages than the traditional ones. The one drawback of this garlic peel-based insect repellent was, that it did not show any expected antibacterial activity when it showed humungous antifungal activity towards most of the fungi tested against the insect repellent such as *Saccharomyces cerevisiae, Aspergillus niger*.

Since the insect repellent formulated in this study is mostly composed of water-based composition the extraction done is most aqueous which is desired in agricultural applications. For utter safety, the continued assessment of ecological toxicity has to be done for every

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product, confirming the safety of humans. For liquid-based nanoemulsion formulations, more systemic in-vivo research has to be conducted like the cytotoxicity, phytotoxicity and genotoxicity and other toxicity tests for both human and animal cell lines have to be conducted to confirm which part of the insect and which life stage of the insect is getting affected (24). The pH and temperature, which is optimum for the formulation have to be verified. Incorporation of all these key attributes will lead to the betterment of the insect repellent or the pesticide production in the time ahead.

Conclusion

This study represents the sample preparation before the extraction process, insect repellent bioassays assessment like the in-vitro bioassay and the toxicity bioassay both in the encapsulate nanoemulsion bead format and the liquid format, where all the components of the formulation will be encapsulated inside the beads. The encapsulated beads with 5th concentration have proven to have the highest repellent percentage of 92% for grain weevils and 87% of fruit flies. The sustainability and stability of the encapsulated beads will exceed with conserved structure due to polymerization and helps in diminishing photosensitivity, evaporation. For the vendors who are suffering from the loss of grains or fruits, this will a boon to all those and quite affordable. Assays can be done further for the proper investigation of the encapsulated beads and to exhibit their impact and potential. These formulations can be infused into many other advanced delivery systems which includes many other components.

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Conflict of Interest

The authors declare no conflicts of interest.

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