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2023203267 24 May 2023

ABSTRACT

The present invention relates to a new formulation and application of the product for insecticide, that is effective in controlling agricultural insects including *Spodoptera litura and Crocidolomia binotalis* and more particularly relates to an insecticidal composition that contains epoxy-linalooloxide, isopropyl 4-methy-3-methylene-4-pentenoate, nerolic acid, and citral such that the insectidal composition is an effective anti-feedant and larvicide but is non-toxic to humans, animals, and the environment.

2023203267 24 May 2023

NEW COMPOSITION FOR INSECTICIDES

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a new formulation and application of the product for insecticide, that is effective in controlling agricultural insects 5 including Spodoptera litura and Crocidolonia binotalis and more particularly relates to an insecticidal composition that contains epoxylinalooloxide, isopropyl 4-methyl-3-rnethylene-4-pentenoate, nerolic acid, and citral such that the insecticidal composition is an effective anti-feedant and larvicide but is non-toxic to humans, animals, and the environment.

BACKGROUND OF THE INVENTION

Crop insects have a destructive impact on crops and the effect often manifests as poor crop yield and heavy economic loss. Spodoptera litura is an extremely serious agricultural insect that is widespread in Malaysia and other regions. It feeds on dicotyledonous crops example, groundnut, and soybean. Currently, there is no efficient means of managing this insect as it is highly resistant to a wide range of insecticides, this implies that the insect multiplies rapidly, Crocidoloia binotalis is a serious insect of cabbage in Asia, Africa, and other tropical regions. It feeds on cabbage, turnip, radish, and other crucifers. Control measures used against this insect include the most commonly used synthetic insecticides.

Although insects have developed resistance to nearly all classes of synthetic insecticides, the synthetic insecticides used in controlling insects pose a lot of problems. They have the potential to significantly alter the ecosystem, for example, through the contamination of waterbodies. They have the tendency to kill natural enemies of insect species and non-target organisms, for example, birds feeding on insecticide-contaminated soil or insects. Excessive use of these synthetic insecticides has also led to the development of insect resistance in crop insects; thus, resulting in an outbreak of insects. Many of these insecticides are also toxic to humans as they are not easily degradable; and thus, concentrate along the food chain.

24 May 2023 2023203267

Accordingly, in search for alternatives to the conventional insecticides, it is an objective of this disclosure to overcome some of the aforementioned disadvantages and find safer and efficient insecticide formulations.

Researchers estimated that in India, extensive feeding by eight larvae of S. litura on a plant can significantly reduce crop yield by about 50% (K. Baskar et al., 2011) whereas Jeyasankar et at. 2011 attributed 10-30% of cultivated crops loss to the feeding activity of this insect. Other similar reports on crop yield losses provoked by this insect include 75-100% in chickpea and 30-50% in soybean (K. Baskar & Ignacinuthu, 2012), 28-100% loss in groundnut has also been reported (Mustaq Ahmad et al, 2007; Kaur et al., 2014).

Currently, synthetic insecticides are applied to agricultural fields for protection against defoliating insects. Whilesome of the insect species are relatively susceptible to the insecticides (Rattan, 2010), other species for example, S. itura have developed resistance to numerous groups of synthetic insecticides such as organophosphates, organochlorines, cabamates, pyretheroids, and the newer chemistry insecticides for example, indoxacarb, spinosad; resulting from the large dependence of crop protection on synthetic insecticide (Munir et al., 2008; Tong et al, 2013). The incessant use of these chemicals in an attempt to maximize crop production eventually leads to development of resistance amongst insect populations, chemical residual effects on harvested food produce, elimination of natural insect enemies, contamination of air and water bodies (de Oliveira et al, 2014), adverse effects on non-target species; domestic and wildlife, species in aquatic systems and soil inhabiting species which play beneficial roles in crop production (Lim et al., 1998). Therefore, the development of novel safer and more effective insecticidal products is highly required to mitigate against the negative impact of synthetic insecticides.

A need exists for an insecticidal composition that kills on contact and controls agricultural insects such as S. litura and C. binotalis by preventing them from feeding on crops, which is non-toxic to humans, soil inhabitants, and the environment. A need also exists for an insecticidal composition that is effective in controlling agricultural insects such as S. litura and C binotalis by killing them, and which is non-toxic to humans, soil inhabitants, and the environment. A need also exists for an insecticidal composition that contains an insecticide made from natural substance that is part of and therefore not harmful to human and the environment. A need also exists for an insecticidal composition that can be used around food, human and animals without worry. There is also need for an insect repellent for use in public health to control disease-transmitting insects such as mosquito. There is also need for an insecticidal composition that can be used in forestry to control insects and promote wildlife, without harming humans, animals, or the environment. In summary, the invention can solve the problems of insect resistance and destructive impact on crops which often manifests as poor crop yield and heavy economic loss. The present invention has relatively low LC_{50}, biodegradable, and environmental friendly.

In an experiment to investigate the insecticidal properties of some essential oils, Ang, Yajun, & Heng (2005) discovered that citral showed strong fumigating toxicity against adult mosquitoes within two hours. Within the same study rutaceae oil also showed considerable activity against the same test insects and GC-MS analysis of the oil revealed that citral was the most abundant compound (69.27%). Apparently, the researchers attributed the activity of mixed-composition of natural organic oil include citral 69.2%, epoxy-linalooloxide (4.12%) and nerolic acid (3,24%), In a later study investigating the anti-inflammatory potentials of, epoxy-linalooloxide was reported as the major active constituent (Sforcin, Amaral, A, Sousa, & Bastos, 2009), however this particular extract has not yet been reported for its insecticidal activity. Nevertheless, lemon grass essential oil has been discovered to have insect repellent properties due to its citral content (Oyedele, Gbolade, Sosan, Adewoyin, & Soyelu, 2002), Although, researchers have reported the presence of epoxy-linalool oxide in different essential oils as a trace or minor constituent (Maham, Akbari, Delazar, 2013; Venkataramani & Chinagounder, 201.2), this study is the first to report the compound as a principal component of a potential insecticidal plant extract. The results of this study indicate that high insecticidal activity due to the abundance of epoxy-linalooloxide.

The insecticidal and anti-feedant properties of LM4 formulation have previously been unknown. Accordingly, in search for alternatives to the conventional insecticides, it is an objective of this research to overcome some of the aforementioned disadvantages and find safer and efficient insecticide formulations. Hence, it would be advantageous to overcome and alleviate these shortcomings with an eco-friendly natural insecticide and anti-feedant especially suited for application in the field of agriculture.

SUMMARY OF THE INVENTION

Accordingly, it is the primary aim of the present invention to provide an eco-friendlv natural insecticide and. anti-feedant especially suited for application in the field of agriculture.

It is yet another objective of the present invention to provide an easily degradable natural insecticide and anti-feedant.

It is yet another objective of the present invention to provide a natural insecticide and anti-feedant which is harmless to non-target organisms and the environment.

Additional objects of the invention will become apparent with an understanding of the following detailed description of the invention or upon employment of the invention in actual practice.

According to one aspect of the present invention there is provided an insecticide and anti-feedant composition, comprising: rutaceae oil; at least one solvent; characterized in that a composition of the rutaceae oil, the solvent, and isopropyl 4 methyl-3-methylene-4-pentenoate forms an active formulation comprising at least one active ingredient of epoxy-linalooloxide, nerolic acid, and citral.

In a related aspect of the present invention there is disclosed an insecticide and anti-feedant composition for controlling agricultural infestation of at least *Spodoptera litura* and *Crocidolomia binotalis* comprising: an effective amount of an extract obtained from Backhousia citriodora (LM) in the presence of a solvent, wherein the extract includes isopropyl 4- methyl-3-methylene-4-pentenoate, epoxy-linalooloxide, citral, and nerolic acid.

The solvent used for extraction of LM can be selected from hexane, water, acetone, chloroform, or a combination thereof.

In a further aspect of the present invention there is disclosed an insecticide and anti-feedant composition for controlling agricultural infestation of at least *Spodoptera litura* and *Crocidolomia binotalis* comprising: an effective amount of an extract

24 May 2023 2023203267

obtained from plant leaves of Backhousia citriodora in the presence of hexane, wherein the extract includes isopropyl 4- methyl-3-methylene-4-pentenoate, epoxy-linalooloxide, citral, and nerolic acid. The extract from Backhousia citriodora in the presence of hexane is a specific isolate LM4 which has a surprising larvicidal activity at low concentrations against *Spodoptera litura* and *Crocidolonia binotalis* effectively preventing the larvae from feeding on crops.

The extract of Backhousia citriodora in hexane includes 23 to 40% of epoxy-linalooloxide, 3 to 15% of isopropyl 4-methyl-3-methyiene-4-pentenenoate, I to 5% of citral, 1 to 10% of nerolic acid and the balance monoterpenoids.

In a further related aspect of the present invention there is disclosed a method of controlling agricultural infestation of at least *Spodoptera litura* and *Crocidolonia binotalis* comprising administering an effective amount of an insecticide and anti-feedant composition having: an effective amount of a plant extract obtained from Backhousia citriodora leaves extracted in the presence of hexane, wherein the extract includes isopropyl 4- methyl-3-methylene-4-pentenoate, epoxy-linalooloxide, citral, and nerolic acid.

Spodoptera litura and *Crocidolomia binotalis* are considered dangerous agricultural insects which have developed resistance to many classes of synthetic insecticides, and insecticides used in controlling *Crocidolomia binotalis* are lethal to living organisms and contaminate the environment. The present invention has found that isolate fraction LM4 obtained from Backhousia citriodora leaves extracted in the presence of hexane has surprisingly powerful larvicidal activity of 100% at 5.00% w/v with a lethal concentration LC_{50} of 1.8% w/v against *Crocidolomia binotalis.* The LM4 fraction also substantially completely inhibits the feeding activity of *Crocidolomia binotalis* larvae representing a good insecticide and anti-feedent.

In the body of the text, the acronym “LM” is a reference to Lemon Myrtle (Backhousia citriodora), a subspecies of Myrtaceae, and LM4 refers to a specific extraction product obtained from lemon myrtle leaves using hexane as the preferred solvent.

2023203267 24 May 2023

BRIEF DESCRIPTION OF THE DRAWINGS

Other aspect of the present invention and their advantages will be discerned after studying the Detailed Description in conjunction with the accompanying drawings in which:

FIG, 1 shows a table of the mean percentage mortality of the different treatments at 1.4% (weight/volume) concentration against Spodoptera litura.

FIG. 2 shows a table of the mean percentage mortality of the different treatments at 1.4% (weight/volume) concentration against Crocidolomia binotalis.

FIG. 3 shows a table of the mean percentage mortality of the LM4 formulation at different concentrations against Crocidolomia binotalis,

FIG. 4 shows a table of the percentage anti-feedant activity of LM4 formulation against Spodoptera litura and Crocidolomia binotalis larvae after 48 hours of treatment.

FIG. 5 shows a table of the ratio of active ingredients in LM4 formulation.

FIG. 6 shows a graph of the larvicidal activity of LM4 formulation at 1.4%(w/v) concentration against 2nd instar Spodoptera litura larvae.

FIG. 7 shows a graph of the larvicidal activity of LM4 formulation at 1.4%(w/v) concentration against 2nd instar Crocidolomia binotalis larvae.

FIG. 8 shows a graph of the increasing larvicidal activity of LM4 formulation at increasing concentration against 2nd instar Crocidolomia binotalis larvae.

FIG. 9 shows a graph of lethal concentration LC_{50} of LM4 formulation against 2nd instar Crocidolomia binotalis larvae.

DETAILED DESCRIPTION OF THE DRAWINGS

In the following detailed description, numerous specific details are set forth in order to provide a thorough understanding of the invention. However, it will be understood by the person having ordinary skill in the art that the invention may be practised without these specific details. In other instances, well known methods, procedures

2023203267 24 May 2023

and/or components have not been described in detail so as not to obscure the invention.

The invention will be more clearly understood from the following description of the embodiments thereof, given by way of example only with reference to the accompanying drawings, which are not drawn to scale.

As used in this disclosure and the appended claims herein, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates or denotes otherwise. Ranges may be expressed herein as form "about" one particular value, and/or to "about" another particular value, When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about, it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

In our finding of producing insecticide and anti-feedant composition. Plant leaves of LM containing organic oil were collected and air dried at room temperature (25°C to 30°C) and powdered by an electric grinder. 1200g leaf powder was soaked at room temperature (25°C to 30°C) in 7 Litres of hexane with occasional shaking for a period of about 72 hours. The resultant supernatant was filtered using a funnel with filter paper. The filtrate was evaporated to dryness at 35C to 40°C (using rotary vacuum evaporator. The remains of the plant materials were extracted with ethyl acetate and methanol sequentially in a similar manner. Masses of the hexane, ethyl acetate, and methanol were weighed and recorded. All extracts were subjected to insecticidal assay and the hexane extract emerged the most effective. The hexane extract (30g) was subsequently coated onto silica gel and subjected to vacuum liquid chromatography (VLC). A sintered glass column 3.5 cm in diameter was inspected for leakages thoroughly cleaned and mounted on the retort stand. The set-up was completed by connecting the column to a vacuum pump. A slurry was prepared by mixing 200g silica gel with four times amount of hexane and stirred to obtain a homogenous mixture free from bubbles. The slurry was poured into the column while tapping gently to facilitate packing, the column was packed to a final height of five inches. Hexane was run through the column several times under vacuum and eventually left overnight to pack the silica. The sample was loaded onto the column and eluted with mixtures of gradient polarity solvents ranging from 100% hexane through different combinations of mobile phase until 80% methanol was reached. Eluents were collected in 200ml volumes or according to observed bands and were pooled to four fractions according to their TLC (Thin Laver Chromatography) profiles monitored on pre-coated silica gel plates (Merck 20 silica gel 60 F254, 0.25mm thick). The developed TLC plates were sprayed with vanillin-sulphuric acid reagent mixture, heated, and visualised under UV (254nm and 366nm) The column was run for one day and the same pressure was maintained throughout. A specific isolate LM4 was obtained by extracting the oil from four fractions of plant extract with similar TLC profile collected over varying combinations of eluting solvents ranging from 100% hexane to 100% chloroform in the following ratios; 100:0, 80:20, 60:40, 20:80, and 0:100. The percentage of solvent is 1 to 99,9%

The larval mortality assay was carried out using leaf dip method. Using a cork borer, fresh cabbage leaves were punched and a leaf disc was dipped individually into various concentrations of the samples in acetone, air-dried and then placed into an assay plate Twenty (20) 2nd instar larvae were introduced individually into the plate and covered with a muslin cloth. Fresh leaves treated with acetone were used as negative control and those treated with B'Green Minyak (a commercial insecticide with neem oil as the active ingredient) was used as positive control. Three replicates were maintained for each treatment with 20 larvae per replicate. Fresh leaves were maintained after 48 hours and the number of dead larvae was recorded every 24 hours, up to 72 hours of treatment. The experiment was conducted under laboratory conditions(27±2°C). Specifically, the larvae were selected randomly from all the members comprising the 2nd instar larvae of Spadoptera litura and Crocidolomia binotalis. It is contemplated that the present invention insecticide can also eradicate the Spadoptera and Crocidolomia genus. The percentage mortality was calculated using Abott's formula represented thus:

$$\text{Corrected \% mortality} = (1 - T/C)*100 \qquad \text{(Equation 1)}$$

Where,

T= Number of living larvae in treatment

C = Number of living larvae in negative control

One way Analysis of Variance was used to determine whether there is significant difference between the means of the treatments, Tukey's Studentized Range (HSD) test at P=0.05 was used to determine which groups were significantly different. All statistical analysis were carried out using SAS 9.4 (English).

The anti-feedant assay is carried out using leaf disc no choice method. Using a cork borer, fresh cabbage leaves were punched and a leaf disc of about 1cm in diameter was dipped individually into various concentrations of the samples in acetone, air-dried and then placed into an assay plate. Twenty(20) 2nd instar larvae were introduced individually into the plate and covered with a muslin cloth. Leaves treated with acetone were used as negative control. and those treated with B'Green Minyak (a commercial insecticide with neem oil as the active ingredient) was used as positive control. Three replicates were maintained for each treatment with 20 larvae per replicate. As this method is carried out to determine the feeding deterrence properties of the extracts, the area consumed by the insects was visually observed after 24 and 48hours. The experiment was conducted under laboratory conditions (27±2°C). Specifically, the larvae were selected randomly from all the members comprising the 2nd instar larvae of Spodoptera litura and Crocidolomia binotalis. The area of leaf consumed by larvae was visually estimated and the percentage anti-feedant activity was calculated according to the formula represented thus:

$$\% \text{ Anti-feedant activity} = (C - T)/(C + T) * 100 \qquad \text{(Equation 2)}$$

Where,

T= Leaf area consumed in treated leaf

C = Leaf area consumed in control

Referring now to FIG. 1, there is shown a table of the mean percentage mortality of the different treatments at 5% (weight/volume) concentration against Spodoptera litura. At 5% concentration, the hexane extract displays a larvicidal activity of 80% against 2nd instar Spdoptera litura larvae after 24 hours of application.

Referring now to FIG. 2, there is shown a table of the mean percentage mortality of the different treatments at 1.4% (weight/volume, w/v) concentration against

Crocidolomia bintalis. Treatment by using hexane solvent showed the highest mortality percentage, 100% against 2nd instar Crocidoloia binotalis larvae after 24 hours of application.

Referring now to FIG 3, there is shown a table of the mean percentage mortality of the LM4 formulation at different concentrations against Crocidolomia bintalis. At 1.4% concentration and after 24 hours treatment against 2^{nd} instar Crocidolomia binotalis, it showed the highest mortality percentage, 100%.

Referring now to FIG. 4, there is shown a table of the percentage anti-feedant activity of LM4 formulation against Crocidolomia binotalis larvae after 48 hours of treatment. The hexane extract displays an anti feedant activity of 100% against 2^{nd} instar Crocidolomia binotalis larvae after 48 hours of treatment.

Referring now to FIG. 5, there is shown a table of the ratio of active ingredients in LM4 formulation. Analysis of LM4 formulation by Gas Chromatography-Mass Spectrometry (GC-MS) identified four major compounds as its active ingredients for the insecticidal activity. The LM4 formulation consists of 23 to 40% of epoxy-linalooloxide ($C_{10}H_{16}O_3$), 3 to 15% of isopropyl 4-methyl-3-methyiene-4-pentenenoate ($C_{10}H_{16}O_2$), 1 to 5% of citral, 1 to 10% of nerolic acid, and 36.5 to 563% of monoterpenoids.

Referring now to FIG. 6, there is shown a graph of the larvicidal activity of LM4 formulation at 1.4% (w/v) concentration against 2^{nd} instar Spodoptera litura larvae. It can be seen that the mortality percentage of 2^{nd} instar Spodoptera litura larvae is gradually increase from 24 hours treatment to 72 hours treatment.

Referring now to FIG. 7, there is shown a graph of the larvicidal activity of LM4 formulation at 1.4% (w/v) concentration against 2^{nd} instar Crocidolomia binotalis larvae. It can be seen that the larvicidal activity of LM4 formulation is constantly displays at high mortality percentage and it displays larvicidal activity of 100% at 72 hours treatment.

Referring now to FIG, 8, there is shown a graph of the increasing larvicidal activity of LM4 formulation at increasing concentration against 2^{nd} instar Crocidolomia binotalis larvae. The results indicate that the larvicidal activity of LM4 formulation increases with the increase of concentration of active extract.

2023203267 24 May 2023

Referring now to FIG. 9, there is shown a graph of the lethal concentration (LC_{50}) of LM4 formulation against 2nd instar Crocidolomia binotalis larvae. It can be seen that the percentage of response increases with the increase of lethal concentration.

While the present invention has been shown and described herein in what are considered to be the preferred embodiments thereof, illustrating the results and advantages over the prior art obtained through the present invention, the invention is not limited to those specific embodiments. Thus, the forms of the invention shown and described herein are to be taken as illustrative only and other embodiments may be selected without departing from the scope of the present invention, as set forth in the claims appended hereto.

2023203267 24 May 2023

What is Claimed is:

1. An insecticide and anti-feedant composition, comprising:

rutaceae oil;

at least one solvent;

characterized in that

a composition of the rutaceae oil, the solvent, and isopropyl 4 methyl-3-methylene-4-pentenoate forms an active formulation comprising at least one active ingredient of epoxy-linalooloxide, nerolic acid, and citral.

2. The insecticide and anti-feedant composition as claimed in Claim 1, wherein the active formulation has 23 to 40% of epoxy linalooloxide.

3. The insecticide and anti-feedant composition as claimed in Claim 1, wherein the active formulation has 3 to 15% of isopropyl 4methyl-3 methylene-4-pentenenoate.

4. The insecticide and anti-feedant composition as claimed in Claim 1, wherein the active formulation has 1 to 5% of citral.

5. The insecticide and anti-feedant composition as claimed in Claim 1, wherein the active formulation has I to 10% of nerolic acid.

6. The insecticide and anti-feedant composition as claimed in Claim 1, wherein the active formulation has 36.5 to 56.3% of monoterpenoids.

7. The insecticide and anti-feedant composition as claimed in Claim 1, wherein the solvent is water, acetone, hexane, chloroform, or a combination thereof.

8. The insecticide and anti-feedant composition as claimed in Claim 1, wherein the percentage of solvent is I to 99.9%.

9. The insecticide and anti-feedant composition as claimed in Claim 7, wherein the solvents hexane and chloroform have ratio of 100:0, 80:20, 10 60:40, 20:80, and 01.00.

10. A use of insecticide and anti-feedant composition in accordance with claim 1 in eradicating agricultural insects.

11. The use of insecticide and. anti-feedant composition as claimed in Claim 10, wherein the agricultural insects are larvae of species podoptera and Crocidolomia.

12. An insecticide and anti-feedant composition for controlling agricultural infestation of at least *Spodoptera litura* and *Crocidolomia binotalis* comprising: an effective amount of an extract obtained from Backhousia citriodora (LM) in the presence of a solvent, wherein the extract includes isopropyl 4- methyl-3-methylene-4-pentenoate, epoxy-linalooloxide, citral, and nerolic acid.

13. The insecticide and anti-feedant composition of claim 12 wherein the solvent used for extraction of LM can be selected from hexane, water, acetone, chloroform, or a combination thereof.

14. An insecticide and anti-feedant composition for controlling agricultural infestation of at least *Spodoptera litura* and *Crocidolomia binotalis* comprising: an effective amount of an extract obtained from plant leaves of Backhousia citriodora in the presence of hexane, wherein the extract includes isopropyl 4-methyl-3-methylene-4-pentenoate, epoxy-linalooloxide, citral, and nerolic acid.

15. The extract of Backhousia citriodora in hexane includes 23 to 40% of epoxy-linalooloxide, 3 to 15% of isopropyl 4-methyl-3-methyiene-4-pentenenoate, I to 5% of citral, 1 to 10% of nerolic acid and the balance monoterpenoids.

16. A method of controlling agricultural infestation of at least *Spodoptera litura* and *Crocidolonia binotalis* comprising administering an effective amount of an insecticide and anti-feedant composition having: an effective amount of a plant extract obtained from Backhousia citriodora leaves extracted in the

2023203267 24 May 2023

presence of hexane, wherein the extract includes isopropyl 4- methyl-3-methylene-4-pentenoate, epoxy-linalooloxide, citral, and nerolic acid.

17. A use of insecticide and anti-feedant composition in accordance with any one of claims 12 to 14 in eradicating agricultural insects including *Spodoptera litura* and *Crocidolonia binotalis* larvae.

2023203267 24 May 2023

Treatment	Mortality (%)		
	24 h	48 h	72 h
Acetone	00.00±00	00.00±00	00.00±00[c]
LM1	00.00±00	00.00±00	00.00±00[c]
LM4	48.33±1.67	78.33±1.67	80.00±2.89[a]
LM5	00.00±00	00.00±00	00.00±00[c]
LM7	00.00±00	00.00±00	00.00±00[c]
Neem Oil	00.00±00	00.00±00	00.00±00[c]

within columns, mean ±SE followed by the same letter do not differ significantly using Tukey's test, $P \leq 0.05$

FIG. 1

Treatment	Mortality (%)		
	24 h	48 h	72 h
Acetone	00.00±00	00.00±00	00.00±00[c]
LM1	00.00±00	00.00±00	00.00±00[c]
LM4	100.00±00	100.00±00	100.00±00[a]
LM5	00.00±00	00.00±00	00.00±00[c]
LM7	00.00±00	00.00±00	00.00±00[c]
Neem Oil	00.00±00	00.00±00	00.00±00[c]

within columns, mean ±SE followed by the same letter do not differ significantly using Tukey's test, $P \leq 0.05$

FIG. 2

Dose (%)	Mortality (%)		
	24 h	48 h	72 h
0.4	00.00±00	00.00±00	00.00±00[f]
0.6	25.00±2.89	25.00±2.89	25.00±2.89[e]
0.8	58.33±1.67	58.33±1.67	58.33±1.67[d]
1.0	75.00±2.89	75.00±2.89	75.00±2.89[c]
1.2	86.67±1.67	86.67±1.67	86.67±1.67[b]
1.4	100.00±00	100.00±00	100.00±00[a]
Acetone	00.00±00	00.00±00	00.00±00[f]
Neem Oil	00.00±00	00.00±00	00.00±00[f]

within columns, mean ±SE followed by the same letter do not differ significantly using Tukey's test, $P \leq 0.05$

FIG. 3

2023203267 24 May 2023

Treatment	Anti-feedant (%)	
	24 h	48 h
Acetone(negative control)	00	00
1.4% LM4 Formulation	100	100
Neem Oil(positive control)	00	00

FIG. 4

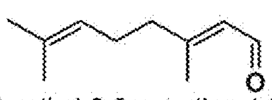
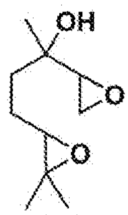
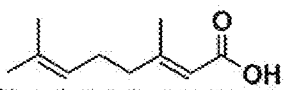
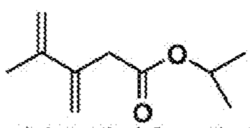
Ratio (%)	Chemical Formula	Molecular Weight (g/mol)	Compound name and structure
1-5	$C_{10}H_{16}O$	152	 3,7-Dimethyl-2,6-octadienal (citral)
23 - 40	$C_{10}H_{16}O_3$	186	 Epoxy-linalooloxide
1-10	$C_{10}H_{16}O_2$	168	 3,7-Dimethyl-2,6-octadienoic acid (nerolic acid)
3-15	$C_{10}H_{16}O_2$	168	 Isopropyl 4-methyl-3-methylene-4-pentenenoate

FIG. 5

2023203267 24 May 2023

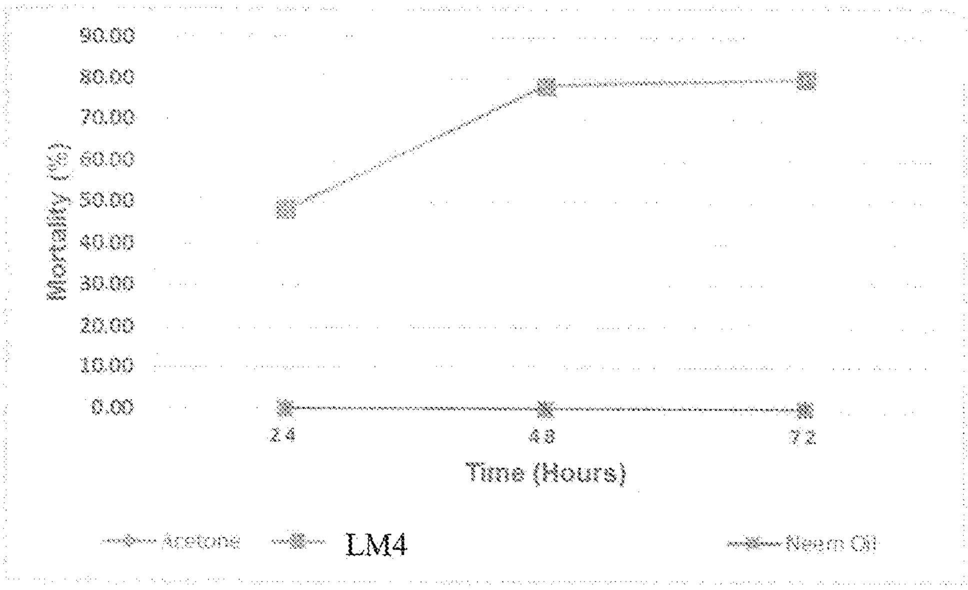


FIG. 6

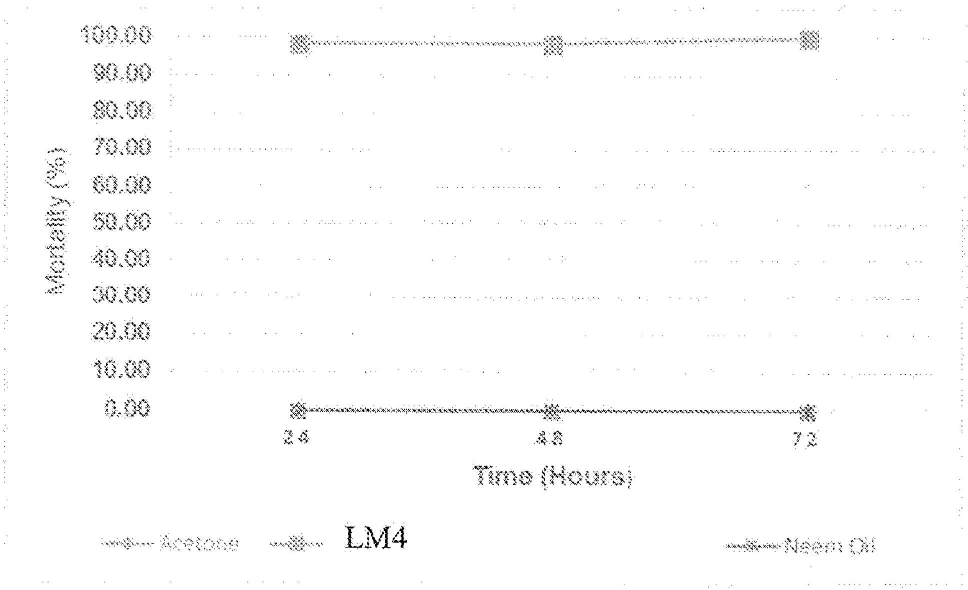


FIG. 7

2023203267 24 May 2023

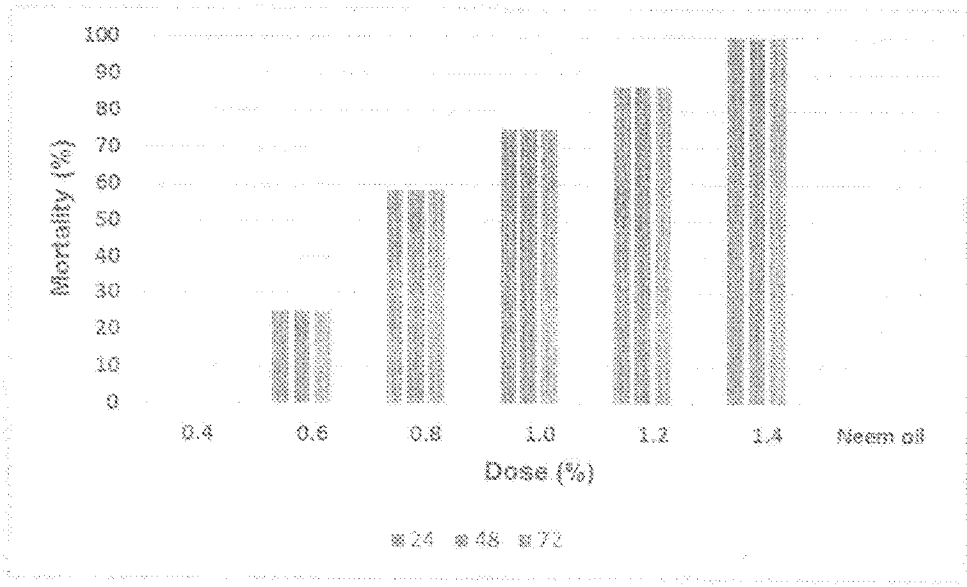


FIG. 8

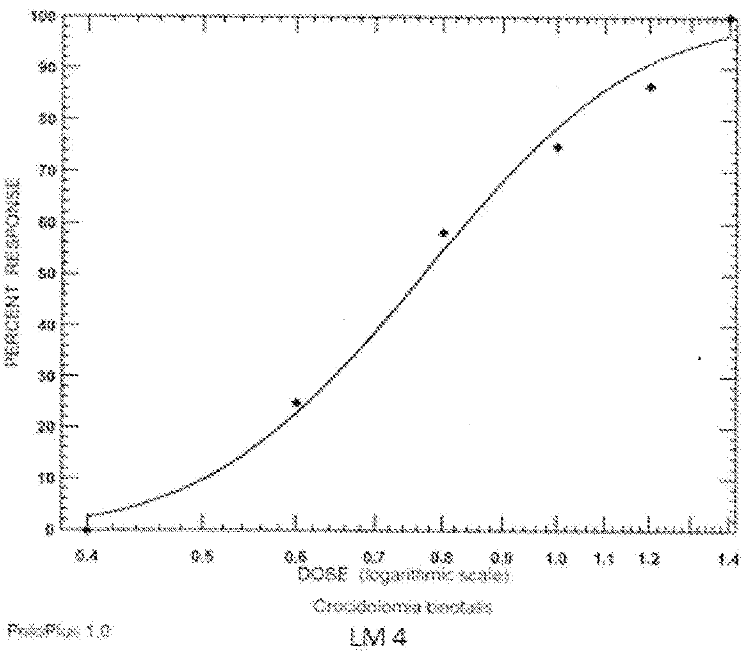


FIG. 9