

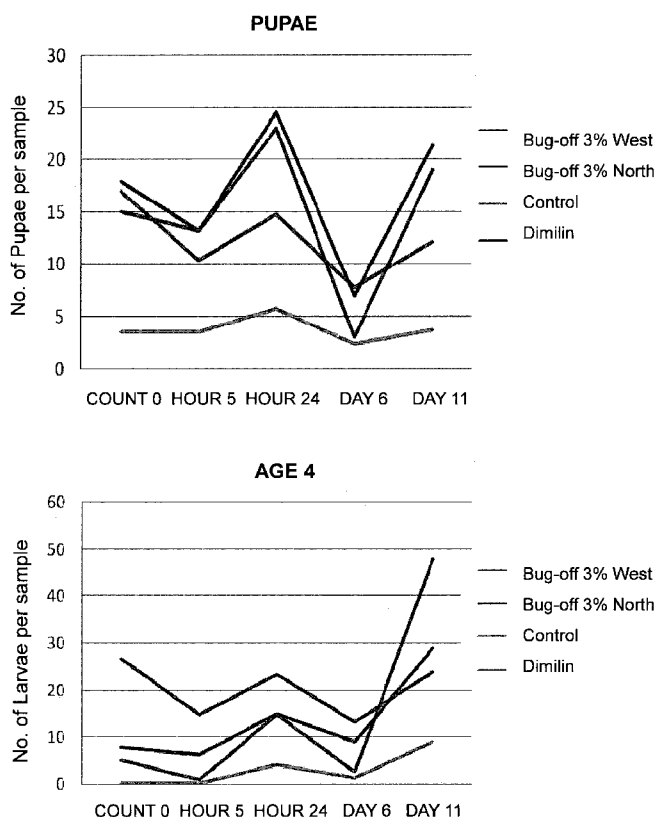


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[Continued on next page]

(54) Title: INSECT-REPELLENT FORMULATIONS

Figure 1



(57) **Abstract:** An insect repellent aqueous formulation comprising a combination of at least two essential oils, citrus peel tincture, and vinegar, adapted for application to an environmental location.



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INSECT-REPELLENT FORMULATIONS

FIELD OF THE INVENTION

The invention generally relates to homeopathy, and more specifically to an all-natural insect repellent.

LIST OF PRIOR ART

5 The following is a list of references which are considered to be pertinent for describing the background and state of the art in the field of the invention. Acknowledgment of these references herein will be made by indicating the number from their list below within parentheses.

- (1) Barnard, D. R. and R.-D. Xue, Laboratory evaluation of mosquito repellents against *Aedes albopictus*, *Culex nigripalpus*, and *Ochlerotatus triseriatus* (Diptera: Culicidae), J. Med. Entomol. **41**(4), 726-30 (2004).
- (2) Fradin, M. S. and J. F. Day, Comparative efficacy of insect repellents against mosquito bites, N. Engl. J. Med. **347**(1), 13-18 (2002).
- (3) Fried et al., U.S. Patent No. 7,201,926.
- (4) Meyhoefer, U.S. Patent Appl. Publ. No. 2006/0057174 A1.
- (5) Bassett, U.S. Patent No. 5,711,953.
- (6) "West Nile Virus (WNV)", Dept. of Health and Human Services, Centers for Disease Control and Prevention, April 18, 2006, available at <http://www.cdc.gov/ncidod/dvbid/westnile/resources/uprepinfo.pdf>.
- (7) "Protection against mosquitoes and other arthropods", Travelers' Health: Yellow Book: Health Information for International Travel, 2005-2006, Dept. of Health and Human Services, Centers for Disease Control and Prevention, available at <http://www2.ncid.cdc.gov/travel/yb/utls/ybGet.asp?section=recs&obj=bugs.htm>.

Each of the above-listed references is hereby incorporated by reference in its entirety, except where its terminology or description may be inconsistent with terminology or description used herein, in which case the present terminology or description supercedes

the disclosure of such reference(s) with respect to such specific terminology or description.

BACKGROUND OF THE INVENTION

Scientists have classified over 1 million species of insects on the earth. Some of these species are responsible for carrying and transmitting diseases to other animals, such as humans.

Some of the best known insect carriers of disease belong to the family Culicidae (mosquitoes). Public concern over the spread of disease by mosquitoes has existed at least since 1898, when Sir Ronald Ross showed that malaria was spread by these insects, but concern has heightened at various times in our recent history, such as in the last decade in connection with the rise of the West Nile virus.

In order to reduce the risks of contracting diseases carried by mosquitoes and other arthropods, public health authorities such as the U.S. Centers for Disease Control and Prevention (CDC) often recommend one or a combination of techniques such as avoidance, use of physical or chemical barriers such as nets or airborne aromatics, treatment of fabrics, and use of insect repellents typically applied to the skin.

The best known and most commonly used insect repellent is N,N-diethyl-3-methylbenzamide or N,N-diethyl-m-toluamide (DEET). Although DEET-containing repellents are considered to be effective in repelling insects such as mosquitoes, with the protective time for the wearer generally directly correlating to the concentration of DEET in the formulations, concerns with the compound's harmful ecological and health effects continue.

Some alternative insect repelling compounds are on the market, but such repellents are often weaker and less effective than DEET in terms of hours of protection. Although citronella oil is a well-known and widely used alternative insect repellent, one study found that 10% citronella oil (Natrapel™) repelled three species of mosquitoes for as little as half an hour and for an average of 2.3 hours between the species tested, as compared with 7% DEET (Skinsations™) and 15% DEET (Off!®) repellents, which were effective for as little as 4.7 hours and an average of 4.8 hours between the species, and for as little as 7.0 hours and an average of 7.2 hours,

respectively.(Reference 1) Other all-natural formulations (Neem Aura™, GonE!™, SunSwat™, Bygone™, Bite Blocker™, MosquitoSafe™, Repel™; see reference (reference 1) for ingredients of each) containing certain essential oils tested in the same study were found to be effective for less than an average of 3 hours across the board, except for a formulation containing 26% oil of lemon eucalyptus (Repel™), which repelled the three species of mosquitoes tested for an average of 7.2 hours. Another study tested four commercially available insect repellent products containing DEET as the active ingredient in concentrations of 4.75% (Off!® Skintastic for Kids™) to 23.8% (Off!® Deep Woods™). The products ranged from a mean of 88.4 minutes of protection for the low-concentration product to a mean of 301.5 minutes of protection for the highest-concentration product.(Reference 2)

The same study also tested several other products containing citronella and other oils. Products containing 5% (Buzz Away™) and 10% citronella (Natrapel™) showed means of 13.5 minutes and 19.7 minutes protection, respectively.(Reference 2) A formulation of 10% citronella and 2% peppermint oil showed a mean protection time of 14 minutes.(Reference 2) A formulation including 12% citronella oil, 2.5% peppermint oil, 2% cedar oil, 1% lemongrass oil, and 0.05% geranium oil (Herbal Armor™) showed similar results to the 10% citronella product (mean of 18.9 minutes protection).(Reference 2) A soybean oil (2%)-based product (Bite Blocker for Kids™) showed a mean of 94.6 minutes of protection.(Reference 2) The authors also performed preliminary studies on Repel™ Lemon Eucalyptus Insect Repellent, and found a mean protection time of 120 minutes

According to the CDC, products registered with the U.S. Environmental Protection Agency (EPA) for use as effective insect repellents which contain DEET and Picaridin (KBR 3023) as active ingredients typically provide longer-lasting protection than other repellents, as shown in references (1) and (2), above.(Reference 6)(Reference 7) However, DEET is toxic when ingested and may cause skin irritation in sensitive persons,(Reference 7) and concerns with its safety persist. Oil of lemon eucalyptus (p-menthane 3,8-diole (PMD)) is also registered with the EPA, and shows effectiveness similar to low concentrations of DEET, according to the same studies.(Reference 6)(Reference 7)

Most insect repellents registered with the EPA have warnings not to use them near food or in waters where fish live, or that they should not be applied to

children.(Reference 3) There is, therefore, a growing awareness of the market for alternative, all-natural insect repellents which are safer for human and animal use than the above non-natural repellents. The U.S. Food and Drug Administration (FDA) classifies certain compounds, including certain essential oils showing some insect
5 repelling activity, as "GRAS", or "Generally Regarded As Safe", compounds. These include citronella oil and D-limonene, a member of the terpene class of compounds.(Reference 3)

Citronella oil and eucalyptus oil (also called oil of lemon eucalyptus; active ingredient: p-Menthane 3,8-diole) are commonly used natural insect repellent
10 compounds, and are also registered for use as such by the U.S. EPA.(Reference 7) Various other essential oils have also been purported to have insect repelling properties, although generally with very limited times of effectiveness, usually being less than one hour. One study concluded that the "inclusion of essential oils in repellent products does not ensure activity against mosquitoes. The basis for incorporating other plant essential
15 oils or extracts in mosquito repellent products is problematic."(Reference 1) Another study found that "currently available non-DEET repellents do not provide protection for durations similar to those of DEET-based repellents and cannot be relied on to provide prolonged protection in environments where mosquito-borne diseases are a substantial threat."(Reference 2) Their results showed that, aside from a soybean oil product, "[a]ll
20 other botanical repellents...tested..., regardless of their active ingredients and formulations, gave very short-lived protection, ranging from a mean of about 3 to 20 minutes."(Reference 2)

U.S. Patent No. 7,201,926 discloses an insect repellent composition requiring citronella oil, D-limonene (1-methyl-4-(1-methylethenyl)cyclohexane), two or more
25 synergists (e.g., N-amyl alcohol, aldehyde C-14, aldehyde C-18), and three or more essential oils, preferably being geranium oil, rosemary oil, and peppermint oil.(Reference 3)

U.S. Patent Application Publication No. 2006/0057174 discloses an insect repellent solution comprising 0.3-0.7% rosemary oil, 0.05-0.3% citronella oil, and 0.1-
30 0.3% table salt.(Reference 4) Optionally, 0.07-0.7% of mint, lemon grass, thyme, and/or wintergreen oils, as well as 7-12% vinegar, may be added to the formulation.(Reference 4) The reference does not disclose data as to length of time of protection from mosquito or other insect bites.(Reference 4)

U.S. Patent No. 5,711,953 discloses an insect repellent comprising 90-95% carrier (e.g., vinegar), 0.5-5% garlic extract, 0.5-5% hot pepper extract, and 0.5% surfactant.(Reference 5)

5 SUMMARY OF THE INVENTION

The present invention provides an insect repellent aqueous formulation comprising the following ingredients: a combination of at least two essential oils, citrus peel tincture, and vinegar.

10 The novel combination of ingredients in the insect repellent formulation of the invention creates a substantially enhanced effect compared to that obtained by using each of the ingredients separately.

The formulation of the invention uniquely combines active ingredients possessing repelling effects on insects (e.g. essential oils, vinegar) with an alcoholic extract of citrus peel which allows effective dispersion of the formulation in the air
15 surrounding the person using the formulation, as well as stabilization.

In a preferred embodiment the formulation further comprises an emulsifier.

In one embodiment the essential oils are selected from the group consisting of lemongrass oil, citronella oil, eucalyptus oil, tea tree oil, lavender oil, geranium oil, clove oil, Cajeput oil and Lemon balm oil. The concentration (volume/volume) of each
20 of the essential oils in the formulation ranges between about 0.1% and about 3%, preferably between about 0.5% and about 2%.

In one embodiment, the citrus tincture is mandarin tincture. The concentration (volume/volume) of the citrus tincture in the formulation ranges between about 0.1% and about 1%, preferably about 0.5%.

25 In one embodiment the vinegar is apple vinegar in a concentration ranging between 1%-10%, preferably between 4% and 6%, and more preferably about 5%.

In one embodiment, the emulsifier is Tween 20. The concentration (volume/volume) of the emulsifier in the formulation ranges between about 0.1% and about 1%, preferably about 0.5%.

30 In a preferred embodiment the insect repellent formulation of the invention comprises a combination of lemongrass oil, citronella oil, tea tree oil, lavender oil, mandarin peel tincture, apple vinegar and Tween 20.

In one embodiment, the formulation of the invention further comprises additional ingredients having insect repelling activity.

By another aspect, the present invention provides an applicator suitable for administration of the formulation of the invention.

5 In one embodiment, the applicator is selected from the group consisting of a pump dispenser suitable for spraying the formulation onto the skin, an applicator stick, or napkins soaked with the formulation suitable for rubbing the formulation on the skin and a sticker soaked with the formulation suitable for attachment onto the skin.

10 In another aspect, the present invention provides a method for protecting individuals from being stung by insects comprising applying the formulation of the invention using an applicator as described above.

In another aspect, the present invention provides a method of preparing an insect repellent aqueous formulation comprising:

- a. Mixing a combination of essential oils with an emulsifier and citrus tincture;
- 15 b. Adding to the mixture water at a temperature of about 40°C and vinegar, wherein the final concentration (v/v) of water is 90% and the final concentration of vinegar (v/v) is 5%;
- c. Mixing for about 2 hours at ambient temperatures using a mechanical stirrer; and
- 20 d. Allowing the mixture to rest prior to administration; the resting period may be for about 24 to 72 hours, preferably for about 48 hours.

DETAILED DESCRIPTION OF NON-LIMITING EMBODIMENTS

The present invention provides a novel all-natural insect repellent formulation which is safe for human and animal use and which is environmentally friendly. The formulation
25 of the invention is a combination of two or more active ingredients, which shows a synergistic effect in terms of length of time of effectiveness in repelling insects.

Referring to insect repellent aqueous formulations disclosed below, as well as to methods for preparation of insect repellent aqueous formulations, it should be noted that
30 such insect repellent aqueous formulations and such methods are also effective in repelling different types of insects, such as (though not limited to) the types of insects enlisted in appendix A.

Additionally, those formulations and methods are further effective for: agricultural uses such as disinfestations of maggots (e.g. of mosquitoes in water bodies), general disinfestations of varmints in agricultural crop and in horticultural crop, etc.

Appendix B includes additional information as well as experimental results,
5 which are incorporated herein.

The present invention is based on the surprising finding that a mixture of an alcoholic extract of citrus peels (tincture) with a combination of plant essential oils in an aqueous solution containing vinegar, provided a synergistic insect repelling effect, exhibited by a prolonged duration of protection from insect bites and stings than that
10 obtain by using each of the ingredients separately.

The insect repellent formulation of the invention may also include an emulsifier and preferably comprises citrus tincture, lemongrass oil, citronella oil, tea tree oil, lavender oil, apple vinegar, and Tween -20. This preferred formulation provides effective repellent activity against insects for a long period of time (i.e. at least about 6-
15 8 hours).

As used herein, an "***insect repellent formulation***" is a composition which repels insects. Repellence may be determined by either the inhibition of insect approach, landing on a target or stinging.

As used herein, the term "***synergistic effect***" means a time of insect repellence
20 achieved with a combination of two or more active ingredients which is longer than the greatest time of repellence achieved for any one of the ingredients when applied to a subject's skin as the sole active ingredient.

As used herein, an "***essential oil***" is any concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oils in the context of the
25 invention may include, without being limited thereto, an essential oil selected from lemongrass oil, citronella oil, eucalyptus oil, tea tree oil, lavender oil, spearmint oil, geranium oil, rosemary oil, Lemon balm oil, peppermint oil, pine needle oil, lavandin oil, cinnamon oil, clove oil, thyme oil, wintergreen oil, cedar oil, lemon oil, grapefruit oil, mandarin oil, tangerine oil, orange oil, citrus oil, lime oil, coriander oil,
30 pomegranate oil, soybean oil, cajeput oil, walnut oil, peanut oil, corn oil, canola oil, sunflower oil, sesame oil, linseed oil, safflower oil, olive oil, etc.

As used herein, "*citrus tincture*" is an alcoholic extract of citrus peel. As used herein, Citrus refers to a genus of fruit comprising, but not limited to, orange, bergamot, grapefruit, lemon, lime and mandarin.

"*Vinegar*" in the context of the invention refers to a sour, aqueous liquid, generally containing about 4%-8% acetic acid, obtained by the acetic fermentation of dilute, aqueous alcohol solutions, e.g. by bacterial fermentation of wine, apple cider or fruit juice.

As used herein, the term "*active ingredient*" means a compound which possesses an insect repelling activity when applied to a subject's skin, e.g. turpentine, pennyroyal.

As used herein, the term "*emulsifier*" (also known as an "*emulgent*") means a substance which stabilizes an emulsion, frequently a surfactant. Non limiting examples include Tween and paraffin oil.

As used herein, the term "*aqueous formulation*" means a water-based composition, wherein the major component of the composition, by volume, is water.

DESCRIPTION OF NON-LIMITING ILLUSTRATIVE EXAMPLES

Materials

The following plant-based materials were each purchased from Perry & Lowe, Hamburg, Germany, and used without dilution: lemongrass oil, tea tree oil, citronella oil, lavender oil. All-natural 5% apple vinegar (95% water) was purchased in Israel at Carmel Mizrachi. Mandarin peel alcoholic extract (tincture) was purchased in Israel.

Formulations

Various formulations comprising each of the above natural substances or their mixtures were assayed for their ability and efficiency in repelling insects.

I. Single active ingredients

Each of the following ingredients was tested as a single component: lemongrass oil, citronella oil, eucalyptus oil, tea tree oil, lavender oil, geranium oil, clove oil, Cajeput oil, Lemon balm and 5% apple vinegar. Each of the listed ingredients was spread as such over an exposed body surface. In addition, preparations containing one essential oil and citrus tincture were also prepared, e.g. lemongrass and mandarin tincture.

II. Single essential oil in a formulation

The formulation was prepared by mixing the active ingredients with a carrier according to the following procedure:

- 5 An essential oil (e.g. lemongrass oil) was mixed briefly using a mechanical stirrer with mandarin tincture, and Tween 20. This was followed by the addition of distilled water heated to 40°C and apple cider vinegar, and the mixture was further stirred for about two hours. The resulting formulation obtained was an emulsion comprising 5% (v/v) cider vinegar, and 90% water.

10

III. Combined essential oils

A combination of the following ingredients was tested. Geranium oil, clove oil, Cajeput oil, and Lemon balm oil were mixed and spread over an exposed body surface.

15 IV. Combined essential oils in a formulation

- Multi-component formulations were prepared by first forming a concentrate comprising a combination of essential oils, mandarin tincture and Tween 20 ("Stock solution"). The concentrate was then mixed for 2 hours, using a mechanical stirrer, with distilled water (heated to 40°C) and natural apple vinegar, to obtain the following v/v
20 ratios in the total diluted formulation:

Stock solution - 5%;

Apple vinegar - 5%;

Water - 90%

- One examined stock solution (Formulation I) included the following ingredients:
25 tea tree oil, citronella oil, lemongrass oil, lavender oil, mandarin peel tincture, and Tween 20 as an emulsifier. The total concentrations of all of the ingredients in the combined, multi-component repellent formulation are provided in Table 1.

Table 1 – Multi-component repellent formulation I

Ingredient	Amount v/v
Tea tree oil	1%
Lemongrass oil	0.5%
Citronella oil	2%

Lavender oil	0.5%
Mandarin peel tincture	0.5%
Apple vinegar	5%
Tween 20	0.5%
Water	90%
Total	100%

Another examined stock solution (Formulation II) included the following ingredients: Geranium oil, clove oil, Cajeput oil, Lemon balm oil, mandarin peel tincture, and Tween 20. The total concentrations of all of the ingredients in the combined, multi-component repellent formulation are provided in Table 1.

Methods

The single or combined formulations were topically applied to exposed (bare) areas of the skin.

Applications of the various formulations was performed using one of the following techniques:

1. introducing the formulations into a pump-dispenser (50 ml) and spraying the formulation onto the skin;
2. attaching a sticker soaked with the formulation onto the skin;
3. saturating fabric napkins with the formulation (by soaking the fabric napkins in the formulation for about 24 hours) and rubbing the napkins on the skin.

Results

Field tests were conducted at three separate locations in Israel having different climates: (1) Neot HaKikar, in the eastern Negev near the Dead Sea; (2) the fish ponds of Kibbutz Maayan Tzvi, on the northern coast of the Mediterranean north of Tel Aviv and south of Haifa; and (3) the orchards of Kibbutz Kabri, on the northern coast of the Mediterranean near the Lebanon border.

Typical species of mosquitoes present at the above three locations were species of the genera *Anopheles* (e.g., *Anopheles gambiae*), *Culex* (e.g., *Culex pipiens*), and *Aedes* (e.g., *Aedes aegypti*). Species belonging to these genera of mosquitoes are known to carry viral and protozoan vectors causing West Nile virus, malaria, Leishmaniasis (*Leishmania major*, *Leishmania tropica*, and *Leishmania infantum*), and Dengue fever. Mosquitoes were present in large numbers at each location. Field tests were conducted in the presence of an entomologist.

Six volunteers (4 men and 2 women) participated in the field experiments. Two tests were performed at each location on consecutive days. On Day 1, the volunteers were exposed without applying any insect repelling preparations and were bitten multiple times on bare arms and legs by mosquitoes within 5 minutes after being in the field.

On the second day at the same locations, each volunteer applied a tested formulation on different locations of the skin, using the sticker, spray or wipes.

The time of repelling mosquitoes ("repellency time") for each of the formulations tested was calculated for each volunteer participant by noting the amount of time elapsed from first exposure to mosquitoes to first mosquito bite received, and the times were averaged for each formulation tested. The repellency times for each single active ingredient formulation tested applied with a stick are indicated in Table 2A.

Table 2A: Average repellency times for each single active ingredient.

Formulation	Time (min.)
Tea tree oil	40
Lemongrass oil	30
Citronella oil	60
Lavender oil	30
Eucalyptus oil	30
Geranium oil	40
clove oil	30
Cajeput oil	40
Lemon balm oil	30
Apple vinegar	90

The repellency times for each combined ingredient formulation tested, applied in the form of each of the sticker, wipes and spray, are indicated in Table 2B. Stickers, wipes and spray all showed approximately the same length of time of repellency. Results were thus independent of formulation type/method of application.

Table 2B: Average repellency times for combined formulations.

Formulation	Time (min.)	Repelling Quality ¹
lemongrass oil and mandarin peel tincture	45	N/A
lemongrass oil, mandarin peel tincture and apple vinegar	135	8
geranium oil, clove oil, cajeput oil, and Lemon balm oil	150	N/A
geranium oil, clove oil, cajeput oil, Lemon balm	315	9

oil, mandarin tincture and apple vinegar		
Tea tree, citronella, lemongrass, and lavender oils, mandarin peel tincture, and apple vinegar	360-480	10

¹The repelling quality is measured at a scale of 1-10, where 1 is hardly any protection and 10 represents total protection.

5 Additional test results

Appendix A and appendix B provide additional test results of a formulation that included Tea tree, citronella, lemongrass, and lavender oils, mandarin peel tincture, and apple vinegar was further tested on several volunteers and on a water canal.

10 Appendix A- results of a test

Methods

A formulation (Herein – the formulation) that included Tea tree, citronella, lemongrass, and lavender oils, mandarin peel tincture, and apple vinegar was further tested on several volunteers and on a water canal was tested.

15 Mosquito larvae were collected from different water sources in the Galilee and northern Israel and were placed in plastic soup bowls with the water of the origin. The bowls were placed on the bottom of a 70x70x70 cm outdoor cage. The cage had four wooden walls (bottom, ceiling, left and the back walls).

Other than the bottom, all wooden walls had a ventilation opening covered with
20 a mosquito mesh. The cage had two glass walls (front and right hand) in addition to the wooden walls. The front glass wall had a 9 cm round opening protected by a mesh sleeve, The cage was positioned in anal day shaded location in Mitzpe Adi I the Galilee, Israel. Dry sugar, 10% sugar water and pitch slices were placed on the bottom of the cage as a nutrition source for the emerged adult mosquito.

25 On September 22007, 120 adult mosquitoes were observed in the cage. About 50% of the mosquitoes were *Culex pipiens* and another 50% were *Culex univittatus*, which are both common in Israel and may transfer the West Nile Virus. About 50% of the adults that were observed that day were females.

At 5: 15 pm the formulation was spread on the left arm of 7 volunteers between
30 the elbow and the hand. The right arm was left unattended.

Between 7: 10 and 7:25 pm, each of the 7 volunteers introduced his/her right (unattended) arm into the cage for 30 seconds, and then his/her left arm for additional

30 seconds. The tester recorded the number of landing mosquito on each volunteer arm during that time (table no. 1). We preformed this inspection in a temperature of 30°C and dusk illumination. We followed this procedure similarly twice more on 8:10 and 9:10 pm. The later inspections differed from the early one only by the source of illumination (artificial rather than sunlight). The temperatures on 8:10 and 9:10 pm were 30° C and 29° C accordingly (table 1).

Results

The average numbers ($\pm$ Standard Error) of landing mosquitoes on the unattended arms in 30 seconds were as follows:

During the first inspection (7:10-7:25 pm, two hours after the repellent was spread) 5.43 (S.E. = ± 0.97) mosquitoes.

During the second inspection (8:10-8:25 pm, three hours after the repellent was spread) 1 (S.E. = ± 0.65) mosquito.

During the third and last inspection (9:10-9:25 pm, four hours after the repellent was spread) 4 (S.E. = ± 1.13) mosquitoes.

During all three inspections (7:10-9:25 pm), no mosquito was landed on none of the treated arms (table no. 1).

Conclusions

According to the results of the control (number of landing mosquitoes on the unattended arms) the formulation was examined with ready to bite mosquito females. The formulation showed high efficiency repelling mosquitoes for as long as four hours. No repellency reduction was observed during the last inspection, four hours after the repellent was spread on the arms, as the formulation remained 100% repellent. Accordingly, it is highly reasonable that the formulation repel mosquitoes for a longer period than 4 hours. The inspected mosquito species (*Culex pipiens* and *Culex univittatus*) are of the most common human biters mosquitoes in Israel and may transfer pathogens like West Nile Virus. The importance of this study on these species is high due to their high levels of distribution and their nuisance and public health hazard effects.

Table number 1: The experimental report of the formulation. The table demonstrates the repellent efficiency protecting the arms of 7 different volunteers from the contact of about 60 *Culex pipiens* and *Culex univittatus* mosquito females.

Time since B.O spread	Time of day	Temp (°C)	# volunteer	Age	Sex	# mosquito landing in 1/2 a minute	
						Untreated arm	Treated arm
2 hours	7:10 pm	30°	1	39	M	8	0
			2	45	M	4	0
			3	45	F	8	0
			4	17	M	6	0
			5	17	M	4	0
			6	17	M	7	0
	7.25pm		7	41	M	1	0
2 hours			Average			5.43	0
			S.E.			0.97	
3 hours	8.10 pm	30°	1	39	M	4	0
			2	45	M	0	0
			3	45	F	3	0
			4	17	M	0	0
			5	17	M	0	0
			6	17	M	0	0
			7	41	M	0	0
3 hours			Average			1.00	0
			S.E.			0.65	
4 hours	9.10 pm	29°	1	39	M	4	0
			2	45	M	1	0
			3	45	F	4	0
			4	17	M	2	0
			5	17	M	5	0
			6	17	M	10	0
			7	41	M	2	0
4 hours			Average			4.00	
			S.E.			1.13	

5

Appendix B – results of another test

A formulation (Herein – the formulation) that included Tea tree, citronella, lemongrass, and lavender oils, mandarin peel tincture, and apple vinegar was further tested on several volunteers and on a water canal was tested.

10

Methods

For the evaluation of the formulation a canal was treated. The canal is characterized by slow running water known as Canal 42. This canal's water contains

many mosquito larvae and pupae (mostly *Culex* sp.). The canal is located between two councils, the town Qiriat-Bialik and the local councils Ma'te-Asher. The water in the canal is running from south to north toward Na'aman stream. The site had no insect control activity for a long period of time and was proved by both councils. The tested
5 canal area was 72m long, 4.6m wide and water depth was approximately 25cm.

Monitoring (manual and visual inspection recorded on video) was preformed from September 18th to October 3rd 2007 as follows:

Sep. 18 - Survey and video recording of the canal for pre-treatment conditions.

Sep. 19 - Survey and video recording of the canal shortly before applying the
10 treatment. Spraying the water surface with 15 liter of 5% liquid agent (covering 4.6xnm).

Sep. 20 - Recording the mosquito larva and pupae level 1 day after treatment.

Sep. 24 - Recording the mosquito larva level 5 days after treatment.

Oct. 3 - Recording the mosquito larva level 15 days after treatment.

15 Each recording activity included total fauna and flora evaluation.

In addition to the aforementioned study, an indoor study examined the mortality rate of *Culex* larvae collected from Canal 42. Additional small study tested the effect of The formulation on the mosquito (*Aedes aegypti*) larvae and pupae in a sea shore rock pool.

20

Results and discussion

The formulation (5% concentration) affected mosquitoes negatively soon after it was sprayed. Within less than half an hour after a few drops of The formulation were
25 add into two test tubes with mosquitoes (*Culex* sp.) all larvae died. In Canal 42, about 26 to 17 mosquito larvae were observed in 70ml water samples. Mosquitoes' levels where examined in this canal about an hour after 15 liter of 5% the formulation were applied over area of nx4.6m. With a resemblance to the indoor results, mosquitoes were found in the treated area in none of the samples. Furthermore, the effect of the
30 formulation was not restricted to the treated area. Since the water in Canal 42 slowly runs northward, the sprayed mosquito control agent was drifted and had a complete negative effect on mosquitoes (100% mortality) at least 200 meters away from the sprayed area. About 400 meters away downstream from the sprayed area no effect of

The formulation was observed. After treating Canal 42 with the formulation, and at the same time when no larvae were found in the treated area, many mosquito larvae were observed in non-treated areas and areas with no drifted control agent (table no. 1). These results suggest that the absence of mosquitoes is a result of the treatment with the formulation and of a quick and effective negative response of the mosquitoes to the formulation.

The effect of the formulation was clearly noticeable long after it was sprayed. Fifteen days after spraying neither mosquito larvae nor pupae was observed in the sprayed area (except for the 5 most southern meters). This result was achieved in spite of the slow running water in the sprayed canal, and nevertheless the formulation effect was not drifted downstream. However, this south to north water flow explain the weakening effect of The formulation in the first most southern 5 meters that was observed 10 days after spraying.

Date	Days after spraying	Mosquitoes in the sprayed area	Mosquitoes outside the sprayed area
Sep. 18	-1	26 larvae per 70ml	Larvae and pupae were observed in the water
Sep. 19	0-an hour	17 larvae per 70 ml	
Sep. 19	0+an hour	0	
Sep. 20	1	0	No larvae 200m northward
Sep. 24	5	0	Many larvae 450m northward
Sep. 28	10	0 (except for the 5 most southern meters)	Many larvae 400m northward
Oct. 3	15	0	Many larvae south to the sprayed area

15

The fauna and flora were observed and found in a similar condition before and after spraying. Both before and after spraying Spur-winged Plover (*Vanellus spinosus*) and Great White Egret (*Egretta alba*) were observed in and next to the water. Adult dragonflies and damselflies were flying above the water and rested on the vegetation before and after spraying. The sprayed area included papyrus (*Cyperus* sp.) plants. After

20

spraying with the formulation no change in plant condition (e.g. turning yellow, leaning down) was observed. After spraying, at the same time when mosquito did not recolonized yet in the sprayed area, two fly species were observed on (a swarm of Ephydriidae) and in (most likely a species of the Stratiomyidae) the water. These observations imply that despite the severe damage that the formulation causing mosquito populations, the aforementioned species are indifferent to this control agent, and that the formulation is probably a specific mosquito control agent. According to these observations, the formulation may be used in a vast variety of environments with little or no fear for environmental negative effects.

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Summarizing some (though not all) significant aspect of the disclosed invention:

1. An insect repellent aqueous formulation including a combination of at least two essential oils, citrus peel tincture, and vinegar.
2. An insect repellent aqueous formulation such as in above item no. 1 further including an emulsifier.
3. An insect repellent aqueous formulation such as in above item no.s 1 or 2 wherein the essential oils are selected from the group consisting of lemongrass oil, citronella oil, eucalyptus oil, tea tree oil, lavender oil, geranium oil, clove oil, Cajeput oil and Lemon balm oil.
4. An insect repellent aqueous formulation such as in above item no. 3 wherein the volume/volume concentration of each of the essential oils in the formulation ranges between about 0.1% and about 3%.
5. An insect repellent aqueous formulation such as in above item no. 4 wherein the volume/volume concentration of each of the essential oils in the formulation ranges between about 0.5% and about 2%.
6. An insect repellent aqueous formulation according to any of the preceding items, wherein the citrus peel tincture is mandarin tincture.
7. An insect repellent aqueous formulation according to any of the preceding items, wherein the volume/volume concentration of the citrus tincture in the formulation ranges between about 0.1% and about 1%.
8. An insect repellent aqueous formulation such as in above item no. 7 wherein the volume/volume concentration of the citrus tincture in the formulation is about 0.5%.

9. An insect repellent aqueous formulation according to any of the preceding items, wherein the vinegar is apple vinegar.
10. An insect repellent aqueous formulation according to any of the preceding items, wherein the volume/volume concentration of the vinegar in the formulation
5 ranges between about 1% and 10%.
11. An insect repellent aqueous formulation such as in above item no. 10 wherein the volume/volume concentration of the vinegar in the formulation ranges between about between 4% and 6%.
12. An insect repellent aqueous formulation such as in above item no. 11
10 wherein the volume/volume concentration of the vinegar in the formulation is about 5%.
13. An insect repellent aqueous formulation such as in above item no. 2 wherein the emulsifier is Tween 20 or Paraffin oil.
14. An insect repellent aqueous formulation such as in above item no. 13 wherein the concentration (volume/volume) of the emulsifier in the formulation ranges
15 between about 0.1% and about 1%.
15. An insect repellent aqueous formulation such as in above item no. 14 wherein the concentration (volume/volume) of the emulsifier in the formulation ranges is about 0.5%.
16. An insect repellent aqueous formulation including lemongrass oil, citronella
20 oil, tea tree oil, lavender oil, mandarin peel tincture, apple vinegar and Tween 20.
17. An insect repellent aqueous formulation according to any of the preceding items, further including additional ingredients having an insect repelling activity.
18. An applicator suitable for administration of an insect repellent aqueous formulation according to any of the preceding items.
- 25 19. An applicator such as in above item no. 18 selected from the group consisting of: a pump dispenser suitable for spraying said formulation onto an individual's skin, an applicator stick suitable for rubbing said formulation on an individual's skin, a napkin soaked with said formulation suitable for rubbing the formulation on an individual's skin and a sticker soaked with said formulation suitable
30 for attachment onto an individual's skin.
20. A method for protecting individuals from being stung by insects including applying to an individual an insect repellent aqueous formulation according to any of the above numbered items 1-17.

21. A method of preparing an insect repellent aqueous formulation including:
- a. Mixing a combination of essential oils with an emulsifier and citrus tincture;
 - b. Adding to the mixture obtained in (a) distilled water at a temperature of about 40°C and vinegar, wherein the final volume/volume concentration of water is
5 90% and the final volume/volume concentration of vinegar is about 5%;
 - c. Mixing the solution obtained in (b) for about 2 hours at ambient temperatures using a mechanical stirrer; and
 - d. Allowing the mixed solution to rest prior to administration.

According to at least some embodiments of the present invention, the compositions or formulations as described herein may optionally be used, or adapted for use, as an insect control measure for environmental locations. Such environmental locations include without limitation any type of outdoor location, including without
5 limitations agricultural fields, landscaped areas, other types of fields and grounds, ponds, lakes, rivers and other bodies of water, outdoor holding pens or coops for livestock, poultry and the like; and also any type of indoor location, including without limitation hothouses, greenhouses, barns, chicken coops or barns, livestock pens and the like.

10 By "insect control" it is meant reducing the numbers of insects, whether as adults or immature forms (including without limitation eggs, pupae and larvae). Such reduction may optionally occur through killing the insects at any stage or more than one of the above stages (wherein each form relates to a different stage in the life cycle of the insect), by reducing the successful reproduction rate and/or by repelling the
15 insects at any one or more stages from the area in which the composition is distributed. Any type of distribution may optionally be used, including without limitation, spraying, spreading, coating, dripping, dissolving and the like, for powder, liquid and foam compositions. Solid compositions may also optionally be distributed in any suitable manner, and may also optionally be dissolved in liquid at the area of
20 distribution. Any suitable carrier may optionally be included to form the composition, for example and without limitation, any type of aqueous carrier, oily carrier, or emulsion (such as oil in water or water in oil emulsions). Producing the various forms of the compositions could easily be performed by one of ordinary skill in the art.

25 A non-limiting list of insects and insect forms against which various embodiments of the present invention may optionally be used are given in Appendix A below. Various tests of the above embodiments of the present invention were performed and are described in Appendix B below.

APPENDIX A

Non-limiting list of insects and insect forms against which various embodiments of the present invention may optionally be used:

- Acaudaleyrodes rachipora (optionally, nymph)
- 5 Aleurocanthus zizyphi (optionally, nymph)
- Aleurolobus marlatti (optionally, nymph)
- Aleurolobus alvinus (optionally, nymph)
- Aleurothrixus floccosus (optionally, nymph)
- Aleyrodes singularis (optionally, nymph)
- 10 Bemisia tabaci (optionally, nymph)
- Dialeurodes citri (optionally, nymph)
- Dialeurolobus rhamni (optionally, nymph)
- Parabemisia myricae (optionally, nymph)
- Paraleyrodes minei (optionally, nymph)
- 15 Siphoninus phillyreae (optionally, nymph)
- Tetraleurodes mori (optionally, nymph)
- Tetraleurodes bicolor (optionally, nymph)
- Tetraleurodes neemani (optionally, nymph)
- Tetraleurodes persae (optionally, nymph)
- 20 Trialeurodes lauri (optionally, nymph)
- Trialeurodes ricini (optionally, nymph)
- Trialeurodes vaporariorum (optionally, nymph)

APPENDIX B**Fumigation of mosquito incubations at 3 sites in central Israel in Spring 2008**

5 Operator's name: Idan (1), Hanan Elraz (2), Avraham Raz (3)

Company name: Luxembourg Industries Ltd (1), S.S Holdings, Bug-Off (2), private instructor (3)

10 **1. Introduction:**

2. Trial objective: evaluation of the efficacy of the Bug-Off and Dimilin preparations in the fumigation of mosquito incubations in three different sites in central Israel.

3. Substances and methods: the series of these trials was performed at 3 sites.

15 **Each site is summarized separately.**

A.

Trial area	Ramat Hakovesh, cooperative settlements water reservoir
Description of water source	A square-shaped reservoir, each side is the size of about 90 meters. Its size is about 1.97 acre. Water depth is over 50 cm.
Trial framework, number of repetitions and their size	4 treatments, each treatment at a side of the reservoir
Meteorological data at the time of application (precipitations and temp.) and abnormal climate phenomena during the trial.	Spraying took place during the morning hours, moderate wind blew during the spraying.

Application date:	16 April 2008
Application method:	Spraying
In application during spraying: sprayer type, reed type, spray volume and pressure:	Motor back sprayer with a gun with an adjustable spray volume conic reed
Dates of evaluations/samples:	16 April 2008 (initial count prior to spraying) 16 April 2008 – 5 hours after spraying 17 April 2008, 22 April 2008
Evaluation method	5 points in a pre-marked area were sampled from each treatment
Sample size	An area of about 4-5 meters along the bank of the reservoir was sampled from each treatment
Initial infestation	Counted prior to spraying – see results

5

The treatments:

No.	The Treatment	The dose (cc, gr./acre)	Remarks
1	Dimilin	50	Dose according to the label is 0.04%
2	Bug-Off+flattener	300 cc in 10 litres of spray (3%) + 30	

3	Bug-Off+flattener	500 cc in 15 litres of spray (3.3%) + 50	
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Explanation of results in Ramat Hakovesh (see Figure 1): this trial consisted of 4 treatments in which an initial count was carried out prior to the application. Each treatment was on a separate bank of the reservoir. The control was low in any case so the different treatments cannot be compared to it. The amount of eggs decreased 5 hours after the application with the Bug-Off treatments. In contrast, Dimilin, as expected, did not lower the amount of the eggs to the same level as the Bug-Off. 24 hours after the application, the amount of eggs was still low, but not in the Dimilin treatment. 6 days after the application there was an increase in the amount of eggs in all treatments and after 11 days there was a decrease whose origin is unclear, but probably not from the substances. A distinct trend of decreasing amounts was not observed after the application. After 6 days a certain decrease was observed in all stages and it is possible that this was as a result of drying a part pf the pool and not as a result of the substance, since 24 hour after the application, an increase was observed in the various stages.

B. Rosh Ha'ayin train station

Explanation of the results at the train station (see figure 2): the incubation level was very high at the initial count, even with an unprofessional eye you could see that the canal was swarming with mosquito larvae. 24 hours after the application there was a significant decrease in all stages. After 11 days there was an increase in the young levels but the amount of eggs and pupae remained very low.

25 C. Tzur Natan pool

Explanation of the results at the Tzur Natan pool (see figure 3): the incubation at the pool was not uniform. The significant incubation concentrated at a specific area in the pool where there was a lot of feculence. Therefore the variance in the tests was not high. Although a certain decrease was apparent after 24 hours from the time of the application, it did not seem to be so in the field, and in any case, an increase takes place after 6 days.

5. Discussion and conclusions

1. The application at the Ramat Hakovesh pool was not representative. It is impossible to determine during application in a pool, be it as large as it may, the efficacy of a number of substances. It was possible to see the movement of the water due to the wind. As a result of this, the substances also moved. Therefore it is not possible to reach decisive conclusions concerning the efficacy of the various substances.

2. In the canal at the Rosh Ha'ayan train station, it was possible to see that the Bug-Off preparation worked efficiently. The area of the canal is 60 SQM (30X2), 25 cm deep. This area is smaller than the area of the canal where the preliminary trial was observed and in which we were given the preliminary data, which was 330 SQO, 25 cm deep. The amount of substance sprayed and its concentration were similar in both trials. According to this calculation, we could have sprayed one fifth of the amount sprayed, meaning spray volume of 3 litres with 90 cc of preparation (3%), and achieve similar results.

3. At the Tzur Natan pool a reduced amount of substance was sprayed according to the calculation of the previous section. In this pool, 0.24 acres in size, about 0.5 meters deep, 500 cc of preparation were sprayed in 15 litre of water. The amount we should have sprayed was 1.35 litre of preparation in a volume of 45 litre of water. It is possible that the unsatisfactory results from this trial resulted from the too low concentration of the Bug-Off preparation in the pool area.

4. The presence of new egg patches after the application at the three sites suggests that there is no rejection of adulthood. There may also be a connection to the concentration or to the minimal amount of substance that should be sprayed in a given area.

In future trials:

A. The quantity of water at the site or the area of the water should be calculated in acres and the appropriate amount of substance should be sprayed.

B. Two different substances or two different concentrations of the same substance should not be sprayed at the same site (as in the Ramat Hakovesh pool).

30

C. The formulation and its concentration should be uniform and pre-determined.

Dates of evaluations/samples:	16 April 2008 (initial count prior to spraying) 17 April 2008, 22 April 2008
Evaluation method	6 points were sampled around the pool

Initial infestation	Counted prior to spraying – see results
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No.	The Treatment	The dose (cc, gr./acre)	Remarks
3	Bug-Off+flattener	500 cc in 15 litres of spraying (3.3%) + 50 flattener	

Preparation details

Preparation Name	Active Ingredient	Description	Amount of active ingredient
Bug-Off		Emulsifier	
Dimilin	Diflubenzuron	Spray powder	25%

5

4. Results (see also Figure 4)

4	Control without spraying		Initial count was low to begin with
5	Bug-Off + flattener	5%+?	This treatment was localized (pouring the solution straight from the bottle along 1-2 meters close to the bank on 17 April 2008
6	Bug-Off + flattener	100% +?	This treatment was localized (pouring the solution straight from the bottle along 1-2 meters close to the bank on 17 April 2008

B.

Trial area	Rosh Ha'ayin Train station
Description of water source	Sewage residue canal delivery institute. Shallow canal (15-20 cm) 1.5-2 meters wide. 30 meters long.
Trial framework, number of repetitions and their size	One treatment with no repetitions.
Meteorological data at the time of application (precipitations and temp.) and abnormal climate phenomena during the trial.	Spraying took place at noon. No wind
Application date:	16 April 2008
Application method:	Spraying
In application during spraying: sprayer type, reed type, spray volume and pressure:	Motor back sprayer with a gun with an adjustable spray volume conic reed. Spray volume was 15 litre for the canal area
Dates of evaluations/samples:	16 April 2008 (initial count prior to spraying) 17 April 2008
Evaluation method	5 points were sampled along the canal
Initial infestation	Counted prior to spraying – see results

No.	The Treatment	The dose (cc, gr./acre)	Remarks
1	Bug-Off+flattener	500 cc in 15 litres of spray (3.3%) + 50 cc flattener	

C.

Trial area	Tzur Natan pool
Description of water source	Sewage pool with an area of about 0.24 acres, 0.5 meters deep
Trial framework, number of repetitions and their size	One treatment with no repetitions.
Meteorological data at the time of application (precipitations and temp.) and abnormal climate phenomena during the trial.	Spraying took place at noon. No wind
Application date:	16 April 2008
Application method:	Spraying
In application during spraying: sprayer type, reed type, spray volume and pressure:	Motor back sprayer with a gun with an adjustable spray volume conic reed. Spray volume was 15 litre for the canal area

5 **Fumigation of mosquito incubations in Kiryat Ata in Summer 2008**

Operator's name: Idan (1), Hanan Elraz (2), Avraham Raz (3)

10 Company name: Luxembourg Industries Ltd (1), S.S Holdings, Bug-Off (2), private instructor (3)

1. Introduction: Mosquitoes are an environmental hazard in Israel. There are currently a number of preparations used to fumigate mosquito incubations: (data from Avraham Raz and the companies' websites)

MLO – Mosquito Larvicidos Oils. An inclusive name for a number of preparations, all based on oil distillations. The best one is Oil 10 by Rimi which costs 11NIS per litre. The dose is 10-12 litre per 0.24 acres (110-132 NIS per 0.24 acre), based on the quantity of herbarium. The preparations are oil-based and spread quickly over water, each drop spreads 13 times its original size. After the application, the larvae cannot rise to the surface of the water and breathe and they die very quickly. After 48 hours the substance breaks down and is no longer effective. Another substance from this group is the pp222 by Tapazol. Probably (illegible) of Oil 10. but in actuality not as effective.

Malotar Spray preparation with a mechanism similar to MLO, but not MLO. Soap-like substance which creates a thin film.

Oscar 100 (emulsifying concentration) by Makhteshim. Contains 100 g/l Novaluron, a preparation regulating the growth of insects (IGR) which disrupts the formation of chitin in the insect's body and thereby damaging the sloughing process. The preparation causes the death of fly maggots and mosquito larvae at the transformation stage from one larvae level to another. Water "application" 0.05-20cm deep – 0.2% (5-20cc in 10 litre spray when spraying 200 SQM. Water 0.1-50cm deep – 0.5% (10-50 cc in 10 litre of water) for spraying 200 SQM. The price is about 200 NIS per litre. Meaning, maximum prices per 0.24 acre in a depth of 20cm (assuming a spraying in a volume of 50 litre per 0.24 acre) – 100 NIS. At a depth of 50cm – 250 NIS. The frequency of spraying – between 2 to 4 weeks according to the incubation potential and the data of the water reservoir and the rate of water addition to the reservoir causing the dilution of the preparation, herbarium, and inlets at the reservoir's edges making fumigation harder.

Cypermethrin – 200 g/l. Unapproved. Contractors sometimes work with them. Price per litre is about 35 NIS. Dose? In any case, it is a cheap treatment, not sure if effective.

2. Trial objective: Evaluation of the "Hadbara Yeruka" preparation for the fumigation of the various levels of mosquitoes in the canal.

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3. Substances and methods:

Trial area	Drainage canal Kiryat Ata
Description of water source	A 3-meter wide canal, 20-30 cm deep. Herbarium 20-30 cm high inside the canal water and on the edges. There is an extremely weak flow at the center of the canal.
Trial framework, number of repetitions and their size	4 treatments. One repetition for each treatment except for the control which had 2 repetitions. The size of each repetition was 200 SQM [(150 length X 3 width, additional 50 SQM due to the tall herbarium).
Meteorological data at the time of application (precipitations and temp.) and abnormal climate phenomena during the trial.	Spraying took place in the morning under conditions convenient for spraying.
Application date:	29 June 2008
Application method:	Spraying
In application during spraying: sprayer type, reed type, spray volume and pressure:	Motor back sprayer with a gun with an adjustable spray volume conic reed. "Hadbara Yeruka" spray volume was 60 litre per 0.24 acre at a pressure of 6 bar. MLO spray volume 12 litre per 0.24 acre (of 100% preparation) at a pressure of 3 bar.
Dates of evaluations/samples:	29 June 2008 (initial count prior to spraying) 29 June 2008 – 5 hours after spraying 1 July 2008, 7 July 2008, 16 July 2008
Evaluation method	5 points were sampled from each treatment by Avraham Raz with a

	sample dipper in a pre-marked area. An area of about 3-4 meters was sampled from each treatment along the bank of the reservoir.
Sample size	An area of about 3-4 meters was sampled from each treatment along the bank of the reservoir.
Initial infestation	Counted prior to spray – see results

The treatments:

No.	The Treatment	The dose (litre/0.24 acre)	Remarks
1	Oil 10 (MLO)	12	Spraying 100% of the substance without diluting in water
2	Yeruka	3	
3	Yeruka	6	
4	Control without spraying	-	

5 4. Results

The results are divided according to stages, since each stage has its own developing scale. Each image shows the development of the stage throughout the trial as each curve describes a different treatment. The two control treatments are represented by the average control treatment (see Figures 5 and 6).

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Explanation of results: The control was good throughout the trial. Meaning, there was no interchange between substances in between treatments. The amount of eggs in the control decreased, but it remained stable throughout the trial. The amount of pupae after 5 hours increased in all treatments. This increase may derive from the time of the count which was at noon compared with the initial count which took place in the morning. MLO behaved as was expected from it. After 5 hours it reduced the

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population. In all stages the peak of its activity was after 3 days. After 9 days it was ineffective as expected.

“Hadbara Yeruka” preparation proved effective. In most stages reduction of infestation was observed even after 5 hours, there was another decrease 3 days later and incubation levels were next to zero. Compared with MLO, the preparation was less effective after 3 days. But after 9 days the preparation maintained a low level of incubation at a dose of 3 litre per 0.24 acre which was more effective than 6 litre. The preparation was no longer effective after 18 days.

Comments on pricing: “Hadbara Yeruka” costs 80 NIS per litre. At a dose of 3 litre the price per 0.24 acre is 240 NIS. Assuming this treatment is effective for a range of at least 10 days, this price is reasonable. MLO whose price is 110 NIS will be effective for days at the most. Taking into account that with MLO the contractor has to enter twice into the given area in order to achieve the same result as “Hadbara Yeruka”, it is likely that he will prefer a more expensive preparation that saves him additional spraying.

Comments on application: The spraying equipment when using “Hadbara Yeruka” has to be at a high pressure in order for the substance to penetrate the depth of the water and mix. This is a disadvantage compared with MLO products which can also be sprayed with a very simple sprayer that has to deliver the substance to the water surface.

5. Discussion and conclusions

1. The “Hadbara Yeruka” preparation is effective at a dosage of 3 litre per 0.24 acre in canals such as the one in which we conducted the present trial. Efficacy should be evaluated in large water reservoirs as well.
2. There does not seem to be an advantage for the high dose of “Hadbara Yeruka”. Lower doses should also be investigated.
3. The application of “Hadbara Yeruka” should be at a high volume 50-60 litre. This may not be a significant problem with contractors equipped with containers adapted for these volumes. The pressure should be high in order for the preparation to penetrate the water well.

WHAT IS CLAIMED IS:

1. An insect repellent aqueous formulation comprising a combination of at least two essential oils, citrus peel tincture, and vinegar, adapted for application to an environmental location.
2. An insect repellent aqueous formulation according to claim 1 further comprising an emulsifier.
3. An insect repellent aqueous formulation according to claims 1 or 2 wherein the essential oils are selected from the group consisting of lemongrass oil, citronella oil, eucalyptus oil, tea tree oil, lavender oil, geranium oil, clove oil, Cajeput oil and Lemon balm oil.
4. An insect repellent aqueous formulation according to claim 3 wherein the volume/volume concentration of each of the essential oils in the formulation ranges between about 0.1% and about 3%.
5. An insect repellent aqueous formulation according to claim 4 wherein the volume/volume concentration of each of the essential oils in the formulation ranges between about 0.5% and about 2%.
6. An insect repellent aqueous formulation according to any of the preceding claims wherein the citrus peel tincture is mandarin tincture.
7. An insect repellent aqueous formulation according to any of the preceding claims wherein the volume/volume concentration of the citrus tincture in the formulation ranges between about 0.1% and about 1%.
8. An insect repellent aqueous formulation according to claim 7 wherein the volume/volume concentration of the citrus tincture in the formulation is about 0.5%.
9. An insect repellent aqueous formulation according to any of the preceding claims wherein the vinegar is apple vinegar.
10. An insect repellent aqueous formulation according to any of the preceding claims wherein the volume/volume concentration of the vinegar in the formulation ranges between about 1% and 10%.
11. An insect repellent aqueous formulation according to claim 10 wherein the volume/volume concentration of the vinegar in the formulation ranges between about 4% and 6%.
12. An insect repellent aqueous formulation according to claim 11 wherein the volume/volume concentration of the vinegar in the formulation is about 5%.
13. An insect repellent aqueous formulation according to claim 2 wherein the emulsifier is Tween 20 or Paraffin oil.

14. An insect repellent aqueous formulation according to claim 13 wherein the concentration (volume/volume) of the emulsifier in the formulation ranges between about 0.1% and about 1%.

5 15. An insect repellent aqueous formulation according to claim 14 wherein the concentration (volume/volume) of the emulsifier in the formulation ranges is about 0.5%.

16. An insect repellent aqueous formulation comprising lemongrass oil, citronella oil, tea tree oil, lavender oil, mandarin peel tincture, apple vinegar and Tween, adapted for application to an environmental location.

10 17. An insect repellent aqueous formulation according to any of the preceding claims further comprising additional ingredients having an insect repelling activity.

18. An insect repellent aqueous formulation according to any of the preceding claims further comprising a carrier.

15 19. The insect repellent aqueous formulation of claim 18, wherein said carrier provides the form of a solid, liquid, powder or foam.

20. The insect repellent of claim 19, wherein said carrier is suitable for distribution of the formulation through spraying, spreading, coating, dripping, dissolving or sprinkling.

20 21. Use of the insect repellent of any of the above claims, for distributing at an environmental location selected from the group consisting of an outdoor location or an indoor location.

25 22. The use of claim 21, wherein said outdoor location includes one or more of agricultural fields, landscaped areas, other types of fields and grounds, ponds, lakes, rivers and other bodies of water, outdoor holding pens or coops for livestock, poultry and other animals.

23. The use of claim 21, wherein said indoor location includes one or more of hothouses, greenhouses, barns, chicken coops or barns, livestock pens and the like.

24. The use of the insect repellent of any of the preceding claims, for insect control.

30 25. The insect repellent of any of the preceding claims, adapted for insect control.

26. An applicator suitable for administration of an insect repellent formulation according to any of the preceding claims.

27. An applicator according to claim 26 selected from the group consisting of: a pump dispenser or spray applicator suitable for spraying said formulation onto the environmental location.

5 28. A method for protecting individuals from being stung by insects comprising applying to an individual an insect repellent aqueous formulation according to any of the preceding claims.

10 29. A method of preparing an insect repellent aqueous formulation comprising:
a. Mixing a combination of essential oils with an emulsifier and citrus tincture; b. Adding to the mixture obtained in (a) distilled water at a temperature of about 40°C and vinegar, wherein the final volume/volume concentration of water is 90% and the final volume/volume concentration of vinegar is about 5%; c. Mixing the solution obtained in (b) for about 2 hours at ambient temperatures using a mechanical stirrer; and d. Allowing the mixed solution to rest prior to administration.

Figure 1

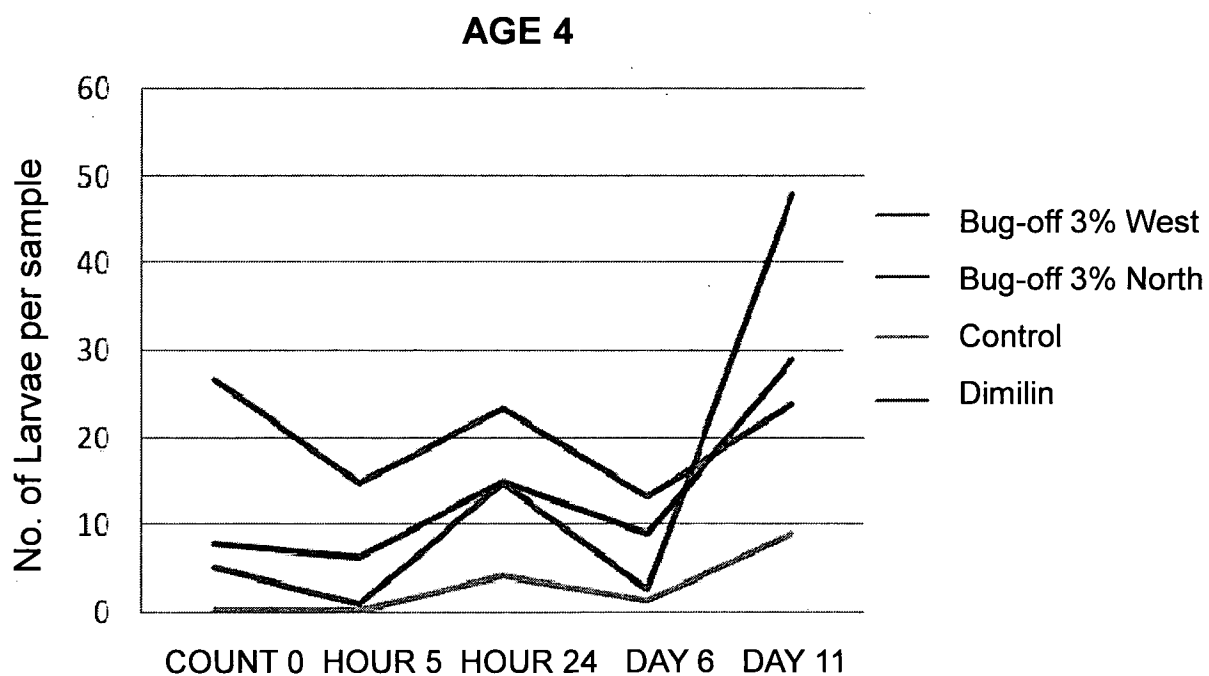
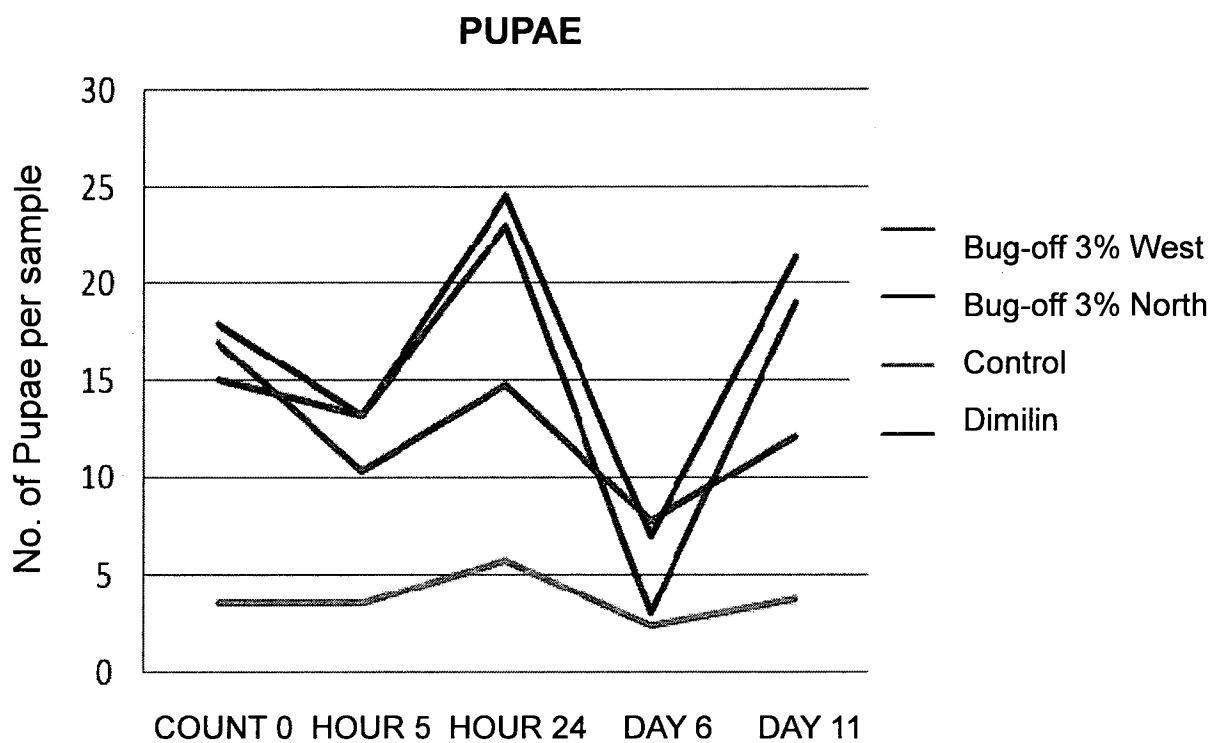


Figure 2

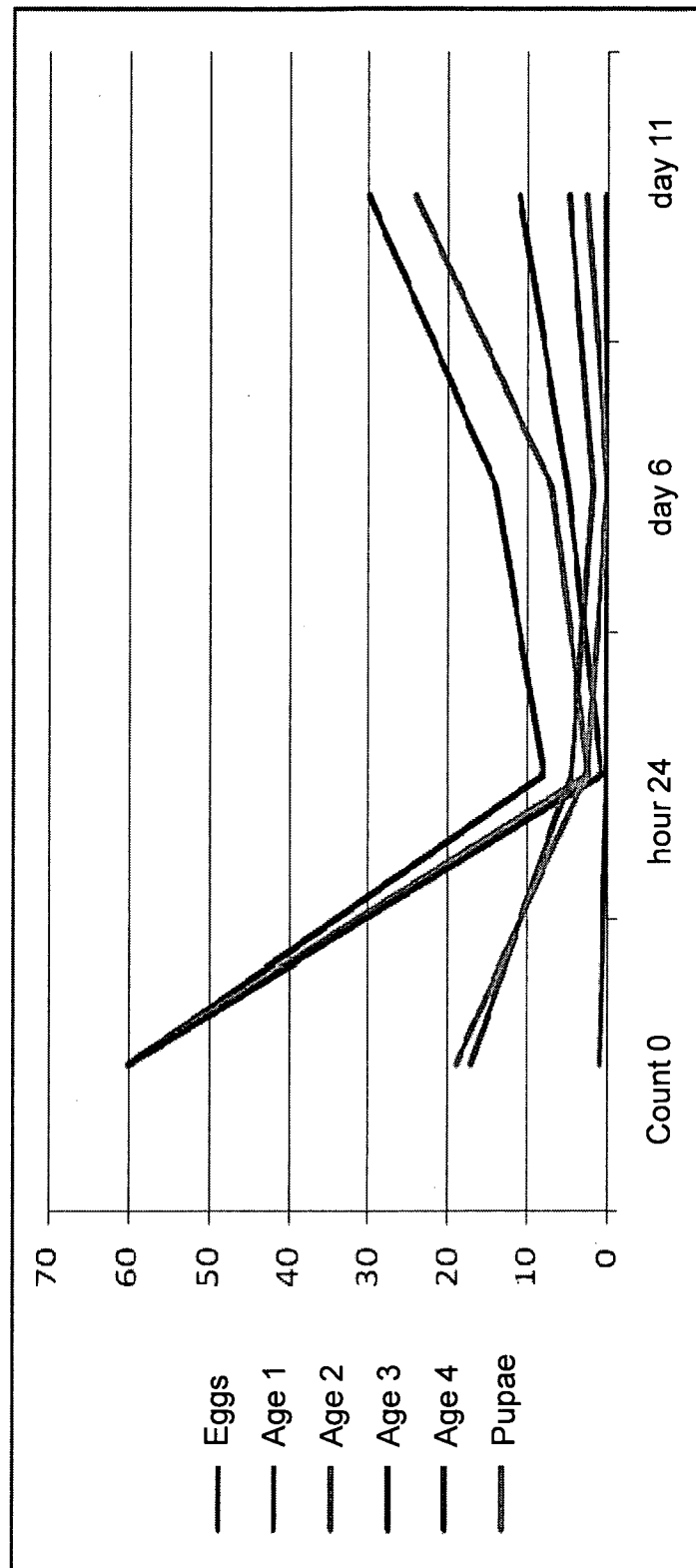


Figure 3

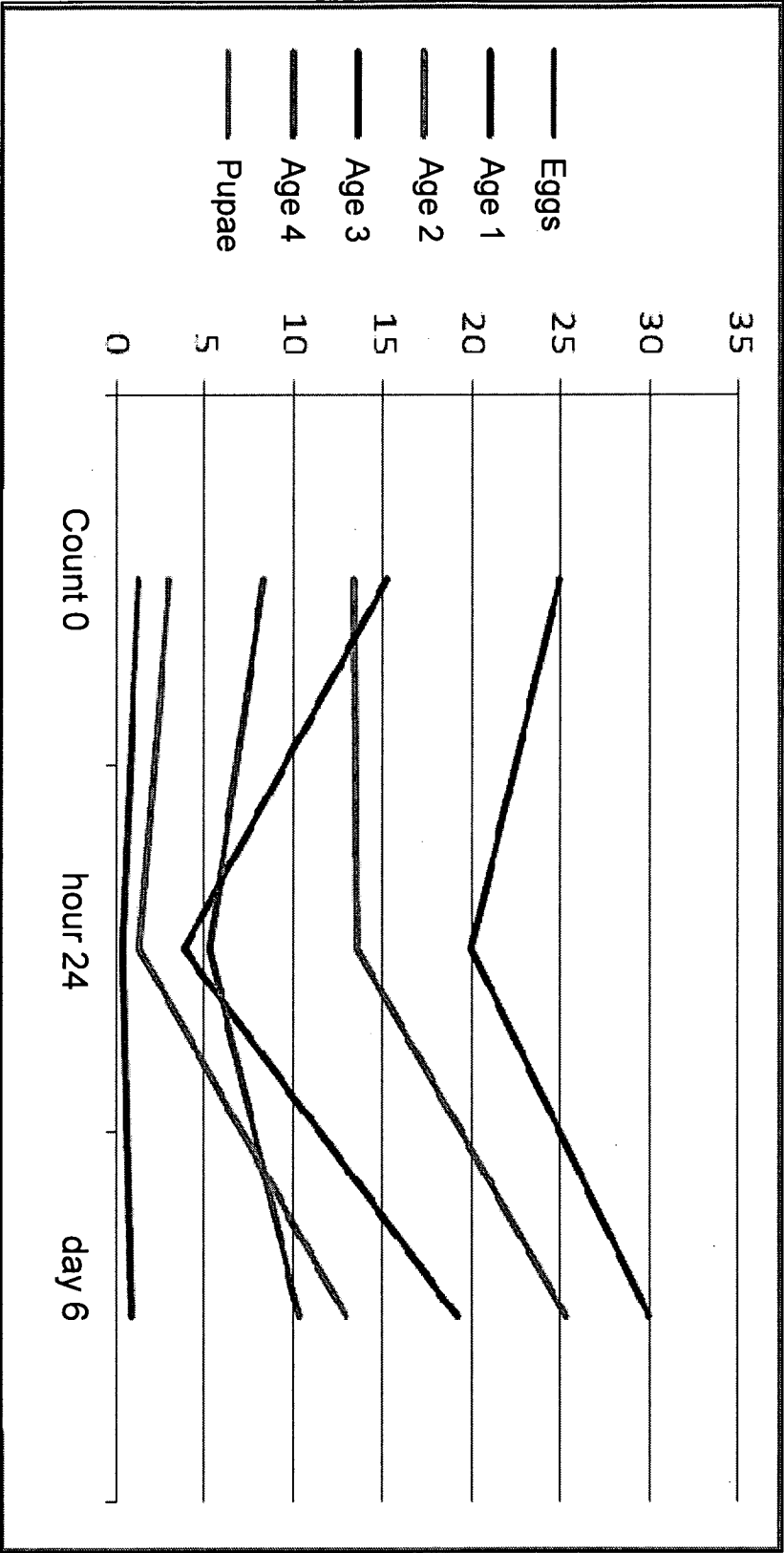


Figure 4

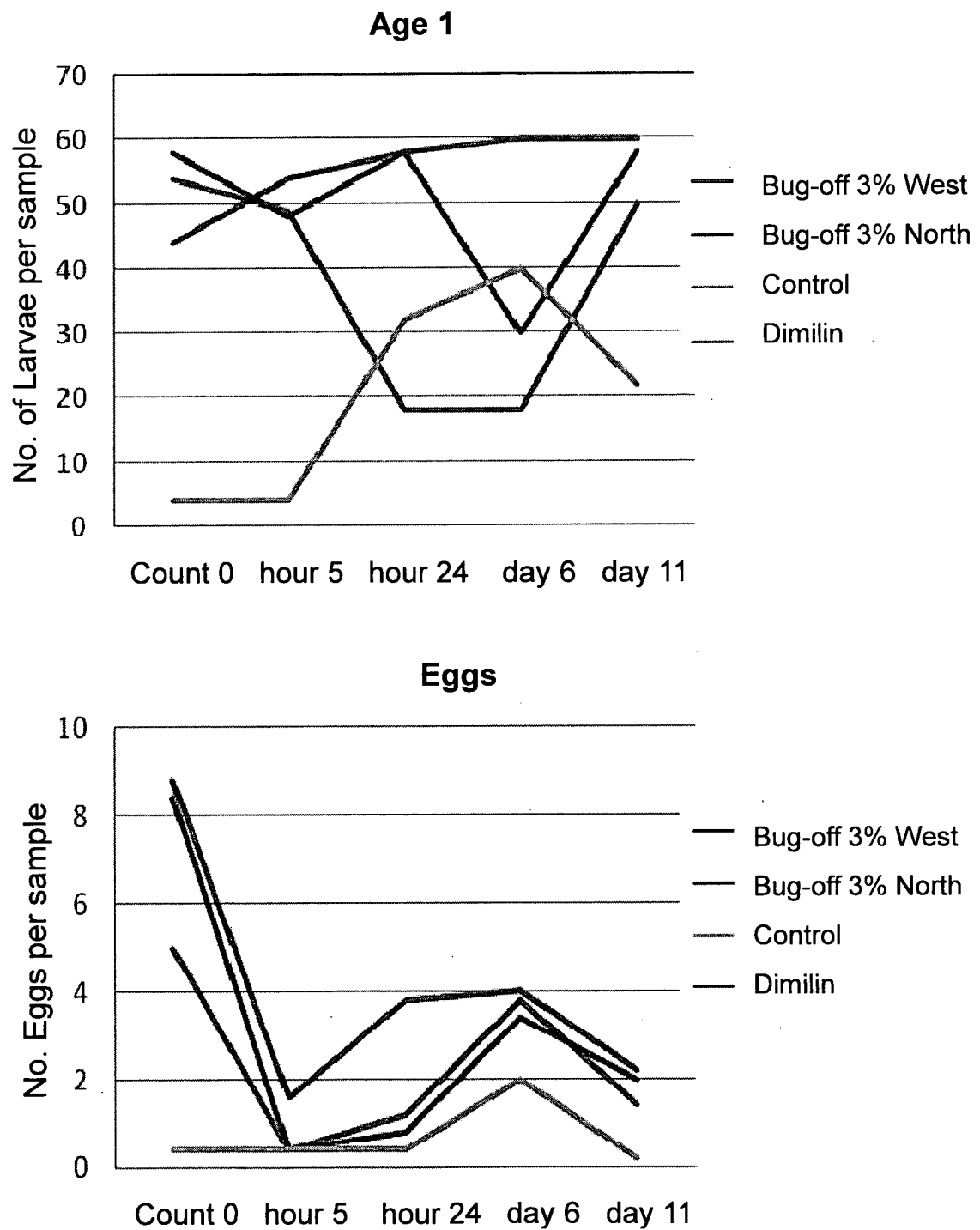


Figure 4 continued

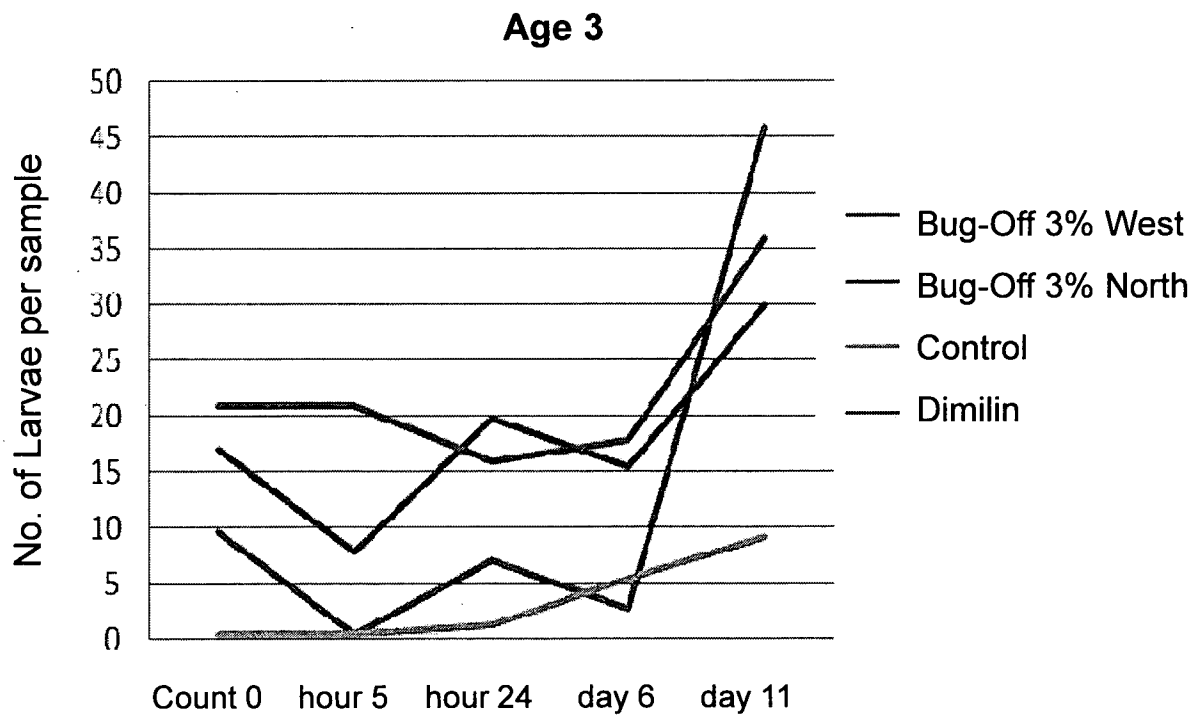
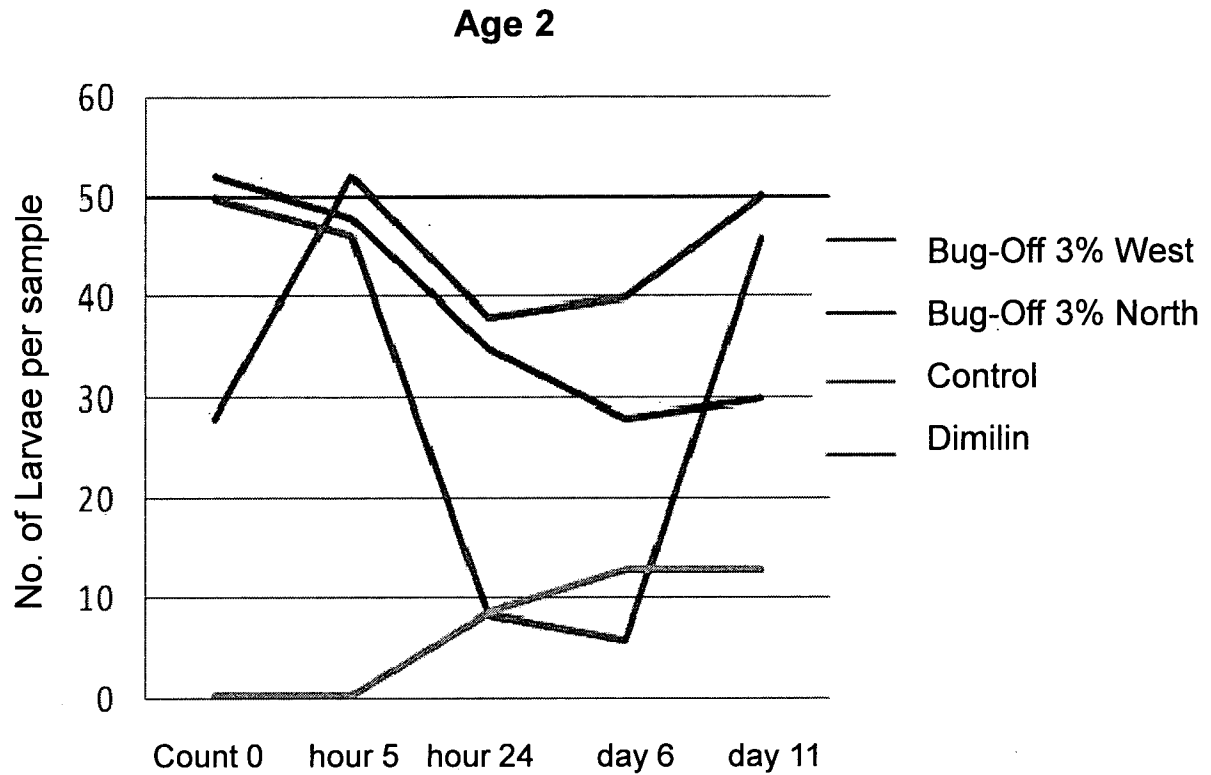


Figure 5

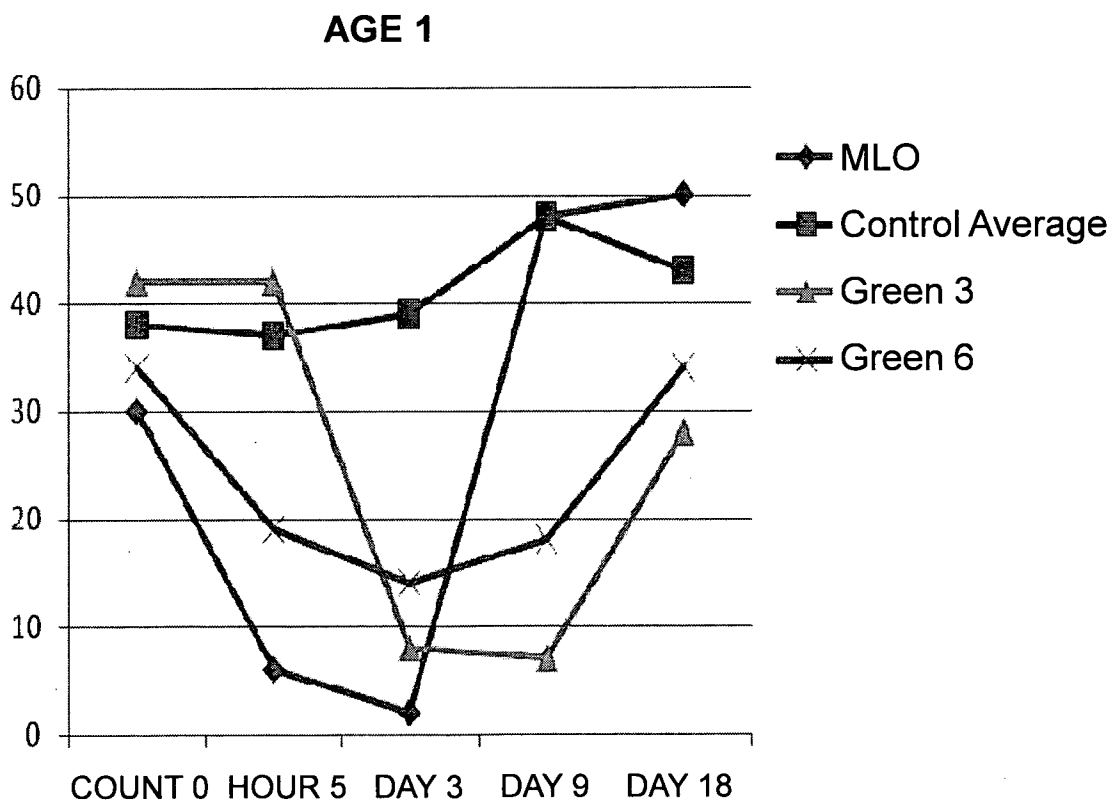
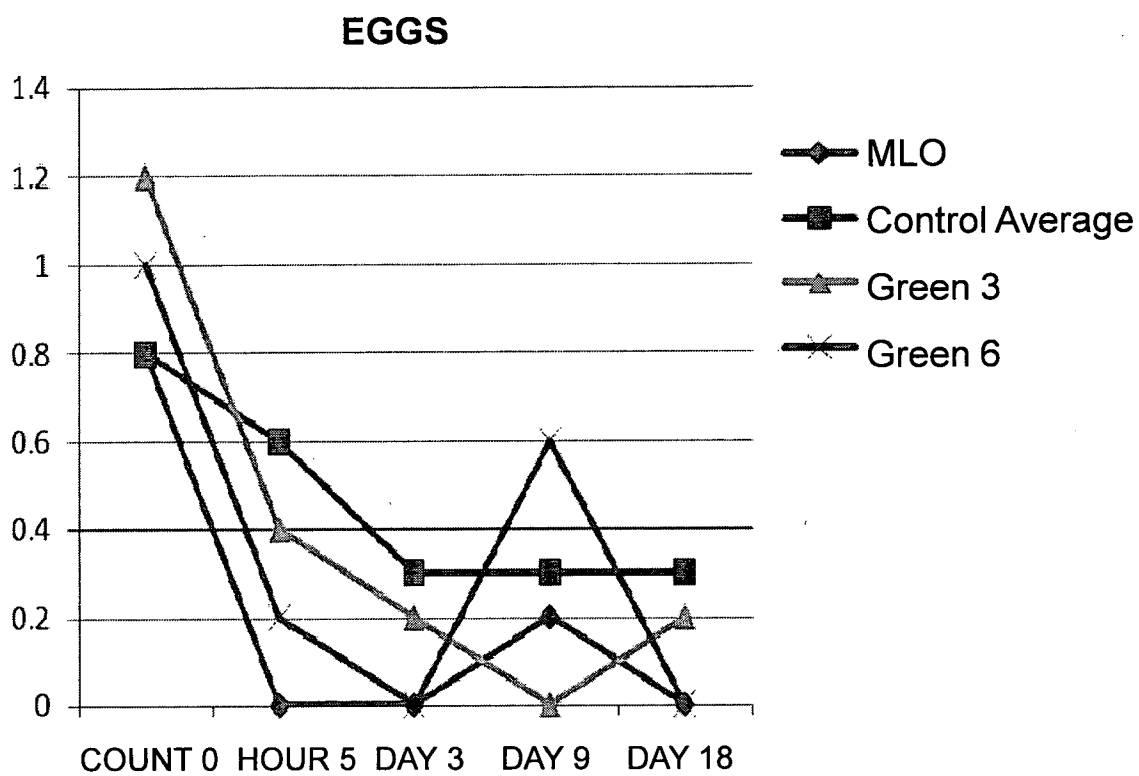


Figure 6

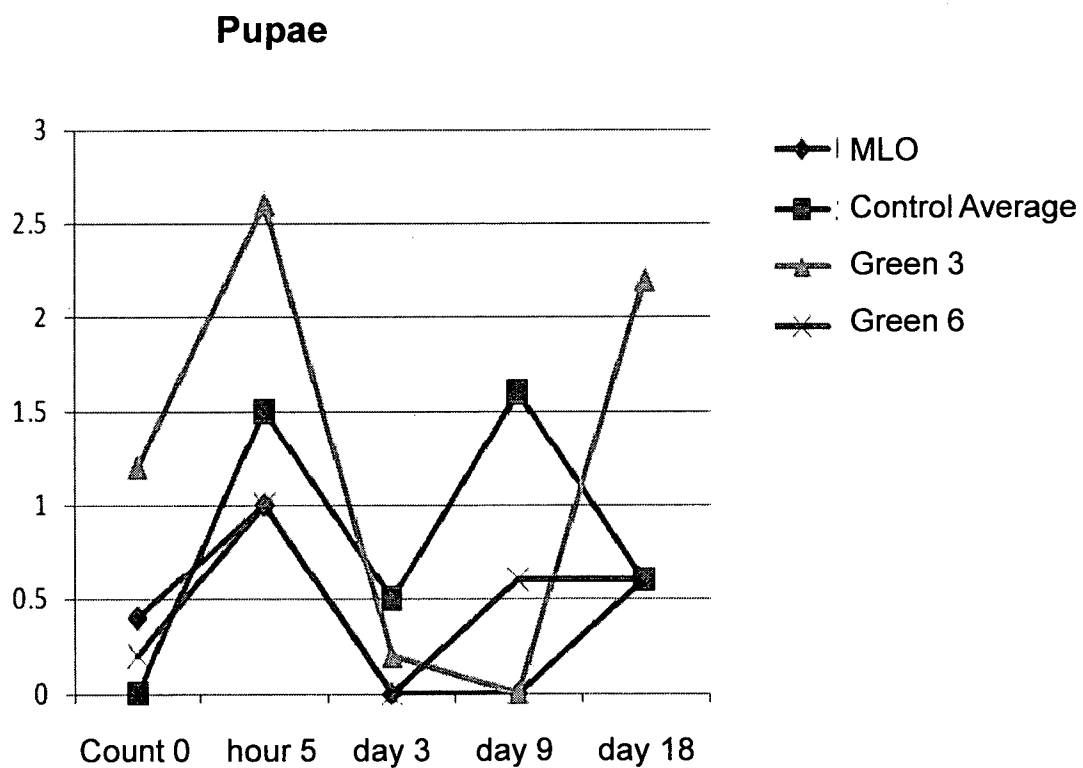
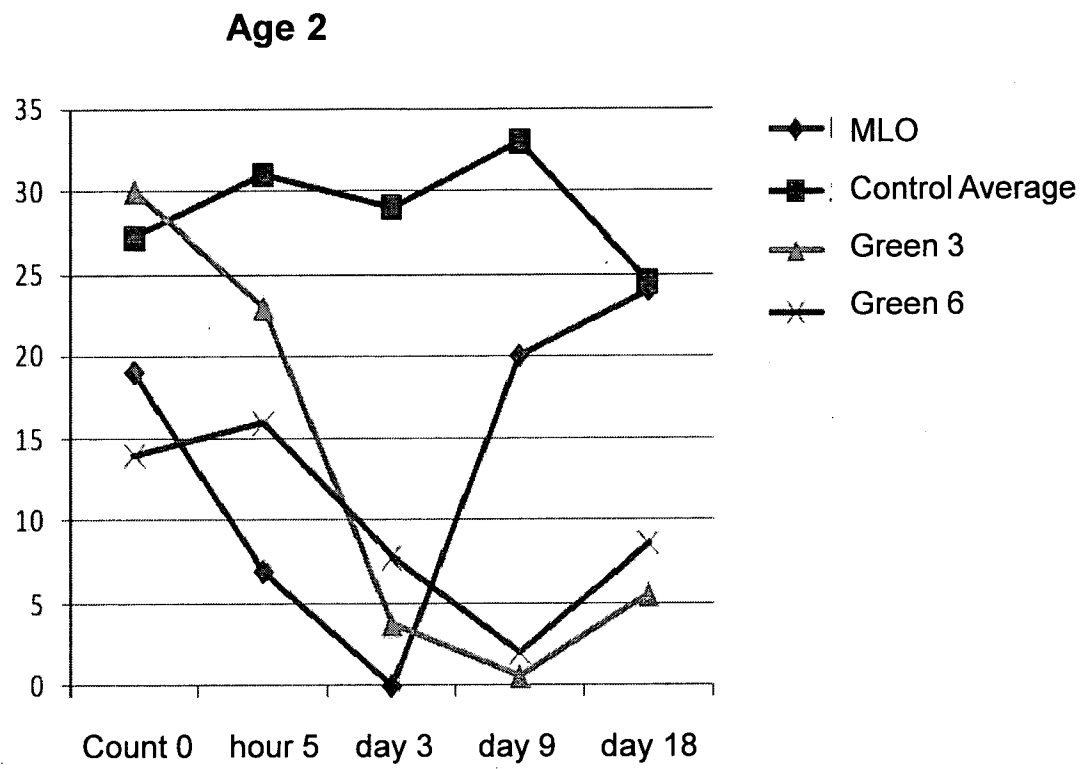
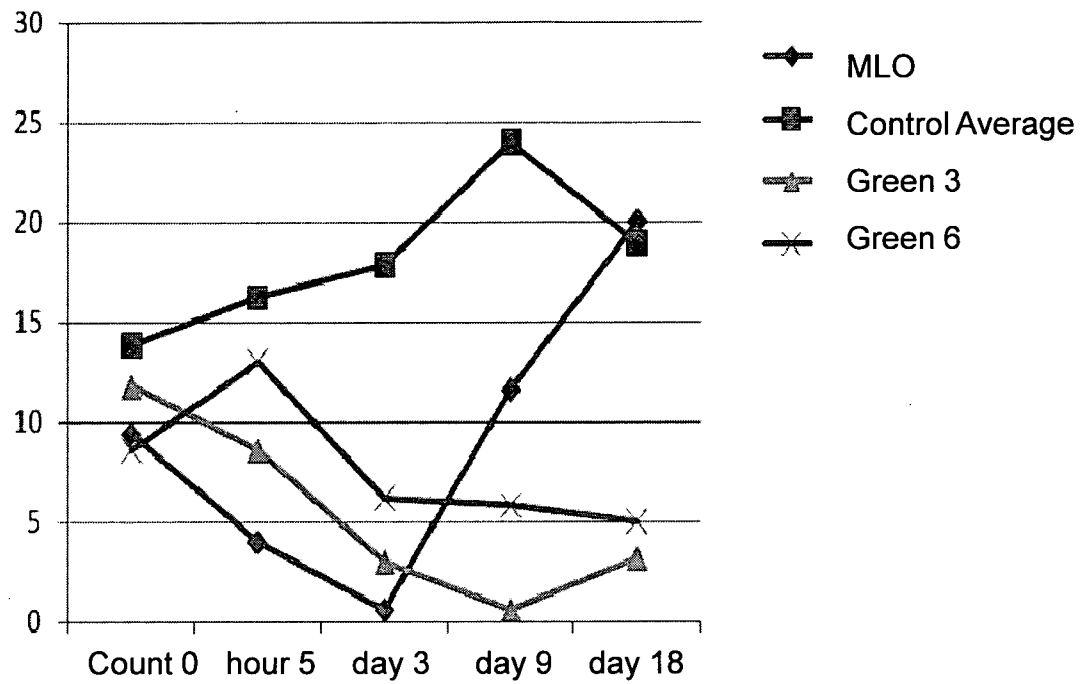


Figure 6 continued

Age 3**Age 4**