

(51) International Patent Classification:

A01P 7/04 (2006.01) A01N 25/04 (2006.01)
A01P 7/02 (2006.01)

(21) International Application Number:

PCT/KE2021/050001

(22) International Filing Date:

04 June 2021 (04.06.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

KE/P/2020/003715

27 August 2020 (27.08.2020) KE

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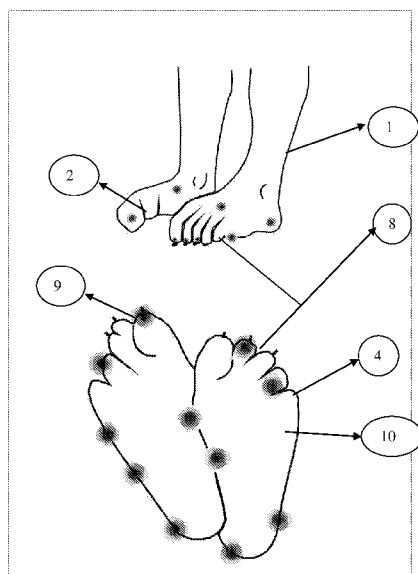
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

(54) Title: BIO-PESTICIDE COMPOSITION FOR KILLING JIGGERS AND OTHER BLOOD FEEDING INSECTS AND ARACHNID PESTS

Fig. 3/4: After Jigger Infestation Treatment



(57) Abstract: Discloses a composition comprising for treating jigger infestation, comprising Nicotine and related compounds present in Nicotiana tobaccum as bio-pesticides and antitungiasis of the jigger flea, Tunga penetrans. A single application of 10% formulation of Nicotine, normicotine, Anabasine, Anatabine, Continine and myosmine in the ratio of 85: 2: 4: 7: 1: 1 respectively in petroleum jelly base of 90% and lemon Eucalyptus oil of 1 g on a jigger (6) infested victim's feet (6) gives 100% pesticide activity within 5-7 days as demonstrated by recovered foot (2B) and jigger scars (8). The said bio-chemicals, alone or in combination with one another and/ or other natural or synthetic pesticides exhibit activity against the jigger flea among other blood feeding insect & arachnid pests of humans and animals. Also discloses a method of formulating the bio-pesticide composition consisting of Nicotine and other biomolecules extracts from Nicotiana tobaccum.

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to the identity of the inventor (Rule 4.17(i))*
- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *of inventorship (Rule 4.17(iv))*

Published:

- *with international search report (Art. 21(3))*
- *with amended claims and statement (Art. 19(1))*
- *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

PATENT DESCRIPTION

Title: Bio-Pesticide Composition for Killing Jiggers and other Blood Feeding Insects and Arachnid Pests

Technical Field

This invention relates to bio-pesticides and more particularly, to a composition suitable for killing and controlling the behaviour of insects and arachnids as well as methods of use of such composition in the field of disease control.

BACKGROUND ON INVENTION

The sand flea (jigger), *Tunga penetrans*, is widely distributed in Latin America, Caribbean and Africa. Jigger transmission from one person to another is not possible but it occurs through the insanitary environment. The jigger flea causes debility in resource-poor communities of developing countries. The flea survives best in sandy and dusty environments. Poverty and powerlessness or inability to do anything about it is the greatest cause of ill health among communities. The Tunga infestation is localized in the foot causing serious difficulty in walking, reducing the infected person's ability to work normally. In endemic areas, prevalence ranges from 15-40% (Julian P. *et al.*, 2009). In Kenya, it is wide spread in Nyanza, Western, Coast, Central and Rift valley regions of the country (Kamau *et al.*, 2014). The female flea lives in close association with the human or animal host, since it needs to burrow into the skin for oviposition for up to 6 weeks (Eisele *et al.*, 2003). The flea is a poor jumper and tends to parasitize the periungual area of the toes (Figure 1), the heels (Figure 2) and the soles of feet. However, embedded sand fleas can be found on almost every part of the body including, hands, neck and the genital region (Heukelbach *et al.*, 2004b).

Human tungiasis, is one of the diseases spread by the flea and is of public health importance, especially in poor communities (Heukelbach *et al.*, 2001; Wilcke *et al.*, 2002; Muehlen *et al.*, 2003; Hermann and Anne, 2013). Another most severe infection spread by fleas is plague, caused by *Yersinia pestis* (Stenseth *et al.*, 2008). Fleas are also known as vectors of murine typhus (endemic typhus, *Rickettsia typhi*), and play a role in the transmission of rural epidemic typhus, *Rickettsia prowazekii* (WHO 1989). More than a decade ago, the flea-borne spotted fever agent *Rickettsia felis* has emerged and can be found throughout the world (Pe´ rez-Osorio *et al.*, 2008). Fleas have also been proven to harbor and sometimes transmit *Bartonella* spp,

including *Bartonella henselae*, the agent of cat-scratch disease (CSD) (Chomel *et al.*, 2006; Billeter 2008; Idir *et al.*, 2010). Jigger flea, *Tunga penetrans*. Jigger infestation has had many socio-economic and health related consequences which translate into most problems in the affected community.

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The Jigger Life Cycle

In the life cycle of a jigger, the larvae live a few centimetres under sand or soil, feeding on organic matter. They are often found inside dwellings with mud floors. The larvae moult to adults about 1mm in size and move to the skin of a variety of mammals including rats, domestic animals and humans. Unlike males, the females burrow into the skin leaving just the tip of their abdomen exposed, thus enabling them to exchange gasses, defecate and mate. The females feed on blood by inserting their proboscis into dermal capillaries. They quickly swell as they become full of eggs which are shed into the environment, after which the females die.

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Effect, Control and Treatment of Tungiasis

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Jigger flea infestation has had many socio-economic and health related consequences which translate into most problems in the affected community. It is estimated that about 2.6 million (6.5%) of Kenyans suffer from tungiasis resulting from the infestation (Ahadi Kenya Trust, 2008). Characteristic complications of extensive tungiasis are atrophy or loss of nails, toe deformations, difficulty in walking and gripping, and lymphadenopathy (Eisele *et al.*, 2003; Feldmeier *et al.*, 2004a; Mazigo *et al.*, 2012). The standard therapy for tungiasis consists of surgical extraction of the embedded parasite under sterile condition, followed by appropriate care of the resulting wound (Brane *et al.*, 2005; Feldmeier *et al.*, 2004a). If the wound is not well handled and dressed, tetanus is a very common secondary infection that normally occurs in such cases and this can even lead to death (Kimani *et al.*, 2012). Topical application of kerosene, plant extracts, chlorophenothane, chloroform, 4% formaldehyde solution, turpentine, and yellow mercury oxide has been used, but without any controlled study (Shatin *et al.*, 1992; Heukelbach *et al.*, 2001). Chemotherapeutic approaches to attempt to kill embedded fleas without mechanical extraction include administration of oral niridazole (Ade-Serrano *et al.*, 1982), thiabendazole (Cardoso, 1981), and Ivermectin (Heukelbach *et al.*, 2004c) none of which is completely effective. Super-infection of the lesions may lead to pustule formation, suppuration, and ulceration (Feldmeier *et al.*, 2002). They can cause multiple abscesses around

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the nail of the fourth and the fifth toe in a child from an endemic area (Hermann and Anne, 2013). In Tungiasis, we find clusters of penetrated sand fleas at the heel in different stages of development on an inhabitant from an endemic area (Hermann and Anne, 2013).

5 Standard therapies, surgical extraction, topical applications and chemotherapy for tungiasis have not been completely effective, sometimes, leading to super-infections that are even more devastating. Various preventive measures including use of biological repellents can be helpful in controlling the flea, however, very little attention if any has been given towards providing lasting reliable solutions to the problem by researchers, especially in Africa.

10 Plants produce many secondary metabolites, of which some are thought to function as direct defenses against pathogens and herbivores by reducing their performance, survival, and reproduction. Numerous plant have chemicals with anti-herbivore properties are classified according to their mode of action (e.g., toxins, anti-feedants, anti-digestive proteins, etc.)
15 (Bennett and Wallsgrove 1994) and have been used in agriculture to control insect pests (Hedin 1991).

Therefore, there is an imperative need for a safe, simple, more effective new treatment and preventive approaches to the menace. These can include the use of chemical repellents and
20 fumigants, which are environmentally friendly and also topical application of anti-jigger natural products with potential to kill jigger *in-vivo*. This has not been adequately explored.

The identification of natural remedies that can terminate the life of the jigger flea *in-vivo* without subjecting the jigger infected victim to any pain such as the ones described herein
25 represent ideal candidates for use in management of the jigger flea among other blood feeding insect and arachnid pests that pose serious threat to human health.

Objects of the Invention

The main object of the present invention is to provide a natural product, comprising Nicotine
30 and other compounds that can control and eliminate jiggers in animals, especially human tungiasis that is common in the tropical world.

The other object of the present invention is to provide a natural product, comprising Nicotine that can control the behaviour of insects and related arthropods, especially blood feeding insects and arachnids.

The other object of the present invention is to teach a method of formulating a natural composition comprising Nicotine for the treating of tungiasis in animals.

SUMMARY OF INVENTION

The present invention describes a bio-pesticide composition for killing blood feeding insects and arachnid pests that comprises a combination of nicotine, normicotine, Anabasine, Anatabine, Continine and myosmine compounds from *Nicotiana tabacum* plant leaf, lemon *Eucalyptus* oil and petroleum jelly base and technically formulated to kill the jigger flea, *Tunga penetrans*.

Also described is the topical mode of application of the bio-pesticide composition on the surface of the victim's feet infested by the blood feeding parasites, *Tunga penetrans*. The powdered dry leaves is mixed with petroleum jelly and Lemon *Eucalyptus* oil and the resulting is mixed to make homogeneous solution. The resultant formulation of the tobacco leaves is applied topically on the jigger infested parts on the body of the victim to provide pesticide activity in a single application on a jigger infested victim.

BRIEF DESCRIPTION OF FIGURES

Figure 1 is a demonstration of a normal feet before infestation of jiggers

Figure 2 shows jigger infested feet in tungiasis stage

Figure 3 shows a recovering feet after jigger infestation treatment

Figure 4 is a schematic flow chart for method of formulating anti-tungiasis

DESCRIPTION OF INVENTION

In a more detailed description, the present invention is based on the characterization of key pesticide constituents in the extracts of *Nicotiana tabacum*, which play an important role in either killing the insect pest or its eggs thereby leading to its complete elimination in the body of the infected victim and surrounding environment.

In **Fig. 1**, the invention demonstrates a normal foot before infestation with the jiggers. The invention shows human leg (1) and foot (2), phalanges (4), nails (3) and toes (5) are clean and healthy.

In **Fig. 2**, the invention demonstrates human feet (2) with jigger infestation (tungiasis). The invention shows a deformed feet (2A) full of female jiggers (6) in different stages of egg maturity. The feet are damaged and cannot enable the victim walk normally. The phalanges or toes (4A) and nails (9) are damaged. The physiology of the leg (1) and entire body are cannot function properly.

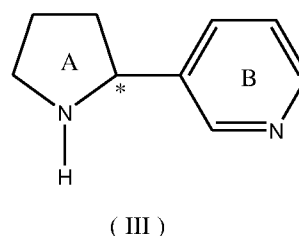
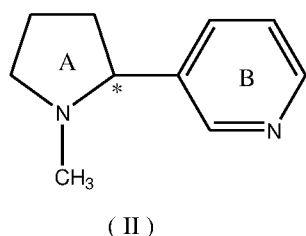
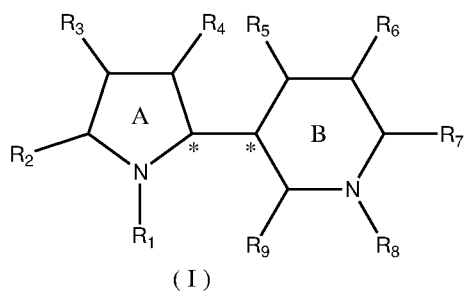
In **Fig. 3**, the invention demonstrates feet (2) of tungiasis victim after treatment with the bio-pesticide composition being taught in the present invention. The Figure shows recovering feet (10) from the deadly tungiasis. It shows rudiment small toe (4) recovering from the infestation. The jiggers are completely done away with after application of the composition and the victim can go about his normal business.

In one embodiment, the present invention describes a bio-pesticide composition for killing blood feeding insects and arachnid pests that comprises a combination of nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine compounds from *Nicotiana tobacum* plant leaf, lemon *Eucalyptus* oil and petroleum jelly base. The seven synthetic blends further comprises 10% - 20% formulation of Nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine in the ratio of 85: 2: 4: 7: 1: 1 respectively in petroleum jelly base. The petroleum jelly base is in the range of 80% - 90% and technically formulated to kill the jigger flea, *Tunga penetrans* as demonstrated in **Fig. 3**.

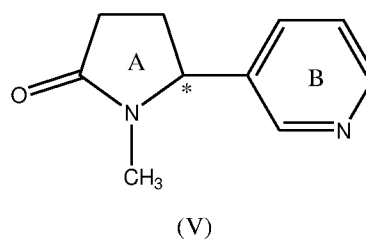
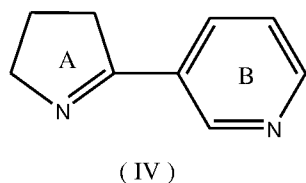
In a similar embodiment, the invention presents a topical mode of application of the bio-pesticide composition on the surface of the victim's feet infested by the blood feeding parasites, *Tunga penetrans*. The powdered dry leaves of about 50 gm is mixed with petroleum jelly of about 100 g and Lemon *Eucalyptus* oil of about 1 g and the resulting is mixed to make homogeneous solution. The resultant formulation of the tobacco leaves is applied topically on the jigger infested parts (on the body including feet) of the victim as in **Fig. 2** to give 100% pesticide activity in a single application on a jigger infested victim within a period of 5-7 days.

In another embodiment according to the present invention, there is provided the use of compounds represented by formulae (I), (II), (III), (IV), (V), (VI), (VII) and (VIII) in killing the blood feeding insect pest, jigger flea *in-vivo* or as it is embedded in the flesh of the affected

person but not limited to the jigger flea, *Tunga penetrans* alone but also applies to the other blood feeding insect and arachnid pests of not only the humans but also other domestic and wild animals:



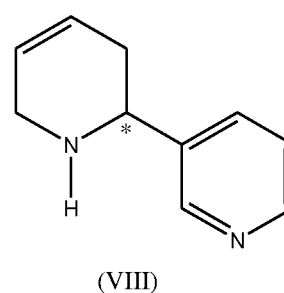
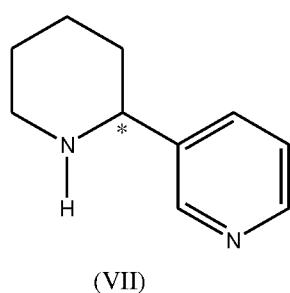
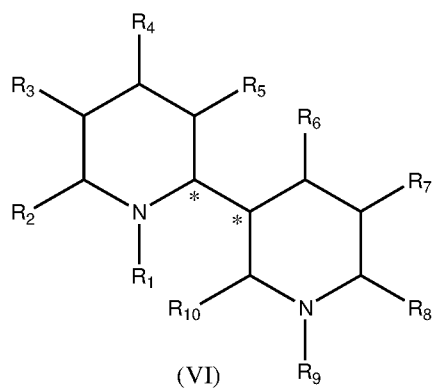
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Where in (I) both rings A and B are either completely saturated in which case carbons marked with asterisk are chiral carbons (stereogenic centers) or completely unsaturated; or Ring A is completely saturated, in which case its asterisk carbon is chiral, while ring B is completely unsaturated; or Ring A is completely unsaturated while ring B is completely saturated in which case its asterisk carbon is chiral; or Rings A and B are partially unsaturated or either A or B is partially or completely unsaturated; when ring A is partially or fully saturated and in which case $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8$ and $R_9 = H$ or an alkyl substituent as in nicotine (II), nornicotine (III), myosine (IV) etc or $R_2 = O$ atom doubly bonded to the carbon of ring A as in cotinine (V).



Where in (VI) both rings A and B are either completely saturated in which case carbons marked with asterisk are chiral carbons (stereogenic centers) or completely unsaturated; or Ring A is completely saturated, in which case its asterisk carbon is chiral, while ring B is completely unsaturated; or Ring A is completely unsaturated while ring B is completely saturated in which case its asterisk carbon is chiral; or Rings A and B are partially unsaturated or either A or B is partially or completely unsaturated; when ring A is partially or fully saturated and in which case $R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9$ and $R_{10} = H$ or an alkyl substituent as in anabasine (VII), anatabine (VIII) etc.

As an important feature of the invention, there is provided the use of any of the above compounds represented by formulae (I), (II), (III), (IV), (V), (VI) (VII) and (VIII) singly or in combinations, with or without other compounds, synthetic or naturally occurring, in protection of humans and other animals both domestic and wild against infestation, bites or diseases spread by blood feeding insects and arachnids pests.

In another embodiment, the invention is meant to include the use of all compositions containing the compounds represented by formulae (I), (II), (III), (IV), (V), (VI), (VII) and (VIII), alone or in blends, in varying proportions, with or without additional constituents, in

killing or deterring blood feeding insects and arachnids pests from feeding on human or animal blood or masking the natural attractive host odor. These may include formulations or concentrates containing diluents, solvents, or dispersing agents, surface-active agents, additives such as antioxidants and UV-screens etc., added for convenience of use or protection of the active agent(s) or its (their) increased effectiveness against the blood feeding insects and arachnids pests of human and other animals. Likewise, it is also to be understood that the invention includes all controlled-release formulations or devices prepared for convenience and/or extending the duration of performance of the individual compounds or blends deployed directly on the body of human and other animals.

Additionally, it is to be understood that the invention is meant to include the use of the said compounds, as formulations or in appropriate devices, with or without additional deterrent or repellent or insecticidal or acaricidal constituents, in control management systems that involve protection of humans or other animals against blood feeding insects and arachnids pests.

In another embodiment according to **Fig. 4**, the present invention teaches of a method of formulating a bio-pesticide composition consisting of Nicotine and other bio-molecules consisting of steps of drying and grounding leaves of *Nicotiana tobacum* plant leaf in water at step (401), extraction of nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine compounds using sequential solvent method using hexane – methyl – ethyl acetate at step (402), mixing of the above compound with lemon *Eucalyptus* oil and petroleum jelly as a base at step (403), melting the jelly at around 45⁰C and allowing it to settle for about 12 hours at step (404), re-mixing the solution by stirring the composition to form a homogeneous solution at step (405), and applying to the victim at least thrice a day for 5 – 7 days as described at step (406).

The invention is illustrated but not limited by the following examples.

Example 1

Jigger Flea killing effect of synthetic blend from *Nicotiana tobacum*

Insecticidal activity of the formulation of *Nicotiana tobacum* plant leaf in Lemon *Eucalyptus* oil plus petroleum jelly and seven synthetic blends [10% formulation of Nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine in the ratio of 85: 2: 4: 7: 1: 1, respectively in petroleum jelly base (90%)] were evaluated against the jigger flea, *Tunga penetrans* in a topical

application on the surface of the guinea pigs feet infested by the blood feeding parasites, *Tunga penetrans*. The powdered dry leaves (50 g) was mixed with petroleum jelly (100 g) and Lemon *Eucalyptus* oil (1 g) and the resulting mixture made homogeneous. The resultant formulation of the tobacco leaves was applied topically on the jigger infested parts on the body of a guinea pig. The pesticide activity of the formulation was monitored for a week after that single application by working out the percentage of jiggers dead each day using the formula: % death = $X/Y \times 100\%$, where X = No. of dead jiggers on a guinea pig, while Y = Total No. of jiggers on a guinea pig (Sum of dead and alive jiggers). Each blend formulation was tested using four different guinea pigs (2 males and 2 females). Control constituted bioassay of the mixture of the petroleum jelly and lemon *Eucalyptus* as well as petroleum jelly and lemon *Eucalyptus* oil singly.

Each of the seven synthetic blends comprised of a 10% formulation of six components [Nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine in the ratio of 85: 2: 4: 7: 1: 1, respectively, which represent the proportions in which the components exist in the plant leaf] and 90% petroleum jelly. The following six (6) components blends were prepared:

Blend (i) was prepared using 6 components in relative proportion present in the typical plant leaf that also exhibited pesticidal activity against the jigger flea, *Tunga penetrans*. These compounds included Nicotine (85 wt%); nornicotine (2 wt%); Anabasine (4 wt%); Anatabine (7 wt%); Continine (1 wt%) and myosmine (1 wt%).

Blend (ii) contained all the compounds of blend (i) except Nicotine.

Blend (iii) contained all the compounds of blend (i) except nornicotine

Blend (iv) contained all the compounds of blend (i) except Anabasine

Blend (v) contained all the compounds of blend (i) except Anatabine

Blend (vi) contained all the compounds of blend (i) except Continine

Blend (vii) contained all the compounds of blend (i) except myosmine

Results

The results of the above experiment (Table 1) show that even though all the six components play a role in killing the jigger flea, nicotine, nornicotine, anabasine and anatabine are crucial components of the blend composition because removal of nicotine led to biggest drop in the number of dead jiggers followed by the removal the remaining three compounds nornicotine, anabasine and anatabine), which led to fairly lower and slower killing effect of the jigger flea embedded on guinea pigs.

Table 1: Percentage Mean of the dead jigger flea embedded on the body of guinea pigs after exposure to various treatments of chemical standards, blends of the standards and *N. tobacum* formulation

%Mean $\pm$ SE							
Treatment	1 st day	2 nd day	3 rd day	4 th day	5 th day	6 th day	7 th day
Control (Petroleum Jelly)	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a
Eucalyptus oil	0.0 $\pm$ 0.0 ^a	0.0 $\pm$ 0.0 ^a	5.0 $\pm$ 1.0 ^b	5.0 $\pm$ 1.0 ^b	5.0 $\pm$ 1.0 ^b	7.0 $\pm$ 3.0 ^b	7.0 $\pm$ 3.0 ^b
<i>Nicotiana tobacum formulation</i>	40.0 $\pm$ 3.0 ^b	50.0 $\pm$ 5.0 ^c	55 $\pm$ 5.0 ^d	64 $\pm$ 2.5 ^d	70 $\pm$ 4.0 ^d	70 $\pm$ 3.5 ^d	80 $\pm$ 5 ^d
Blend (i)	80.0 $\pm$ 4.0 ^c	95.0 $\pm$ 5.0 ^d	97.0 $\pm$ 0.5 ^e	100 $\pm$ 0.0 ^f	100 $\pm$ 0.0 ^g	100 $\pm$ 0.0 ^f	100 $\pm$ 0.0 ^f
Blend (ii)	35.0 $\pm$ 4.0 ^b	36.0 $\pm$ 1.7 ^b	38 $\pm$ 0.09 ^c	44 $\pm$ 2.5 ^c	48 $\pm$ 2.3 ^c	48 $\pm$ 1.5 ^c	50 $\pm$ 2.5 ^c
Blend (iii)	75.0 $\pm$ 2.0 ^c	87.0 $\pm$ 5.0 ^d	90.0 $\pm$ 2.5 ^e	92 $\pm$ 1.0 ^e	95 $\pm$ 2.5 ^e	98 $\pm$ 1.6 ^e	100 $\pm$ 0.0 ^f
Blend (iv)	72.0 $\pm$ 3.0 ^c	80.0 $\pm$ 4.5 ^d	86.0 $\pm$ 3.5 ^e	88 $\pm$ 2.0 ^e	91 $\pm$ 2.0 ^e	95 $\pm$ 3.5 ^e	95 $\pm$ 2.5 ^e
Blend (v)	78.0 $\pm$ 3.0 ^c	90.0 $\pm$ 5.5 ^d	97.0 $\pm$ 2.5 ^e	100 $\pm$ 0.0 ^f	100 $\pm$ 0.0 ^g	100 $\pm$ 0.0 ^f	100 $\pm$ 0.0 ^f
Blend (vi)	75.0 $\pm$ 4.0 ^c	84.0 $\pm$ 3.5 ^d	90.0 $\pm$ 4.5 ^e	98 $\pm$ 2.0 ^f	100 $\pm$ 0.0 ^g	100 $\pm$ 0.0 ^f	100 $\pm$ 0.0 ^f
Blend (vii)	74.0 $\pm$ 4.0 ^c	82.0 $\pm$ 3.5 ^d	88.0 $\pm$ 4.5 ^e	96 $\pm$ 2.0 ^f	98 $\pm$ 1.5 ^f	100 $\pm$ 0.0 ^f	100 $\pm$ 0.0 ^f

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Example 2

Pesticide activity of various dilutions of the synthetic blends on guinea pigs infested with the Jigger

Each of the seven (7) synthetic blends comprising of a 10% formulation of six (6) constituents [Nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine in the ratio of 85: 2: 4: 7: 1: 1, respectively, representing the proportions in which the components exist in the plant leaf] and 90% petroleum jelly was tested for pesticide activity against the jigger flea, *Tunga penetrans* as a topical application on the guinea pigs feet infested by the flea. Blend (i) was prepared using all the six (6) components listed above in relative proportion present in the typical plant leaf that also exhibited pesticidal activity against the jigger flea, *Tunga penetrans* as earlier shown in table 1 results of example1. These compounds included Nicotine (85 wt%); nornicotine (2 wt%); Anabasine (4 wt%); Anatabine (7 wt%); Continine (1 wt%) and myosmine (1 wt%).

Blend (ii) contained all the compounds of blend (i) except Nicotine.

Blend (iii) contained all the compounds of blend (i) except nornicotine

Blend (iv) contained all the compounds of blend (i) except Anabasine

Blend (v) contained all the compounds of blend (i) except Anatabine

Blend (vi) contained all the compounds of blend (i) except Continine

Blend (vii) contained all the compounds of blend (i) except myosmine

Insecticidal activity of each blend was evaluated at four levels of dilutions (5, 10, 15 and 20% w/w) of the total blend composition weight in comparison with weight of petroleum jelly. That is a 5% blend components mixed with 95% petroleum jelly, 10% blend components plus 90% petroleum jelly, 15% blend components plus 85% petroleum jelly and 20% blend components plus 80% petroleum jelly. Four replicates of each diluted formulation of the synthetic blends were tested. Each blend formulation dilution was tested using four different guinea pigs (2 males and 2 females) to generate four replicates. The control experiment involved treatment of the jigger infested guinea pigs with petroleum jelly, which was used in formulation of the synthetic blends.

The pesticide activity of each of the formulations was monitored for a week after a single application by working out the percentage of jiggers dead. The activity of the formulations was monitored for a week after the single topical application by working out the percentage of jiggers dead each day using the formula: % death = $X/Y \times 100$, where X = No. of dead jiggers on a guinea pig, while Y = Total No. of jiggers on a guinea pig (Sum of dead and alive jiggers).

Results

Table 2 summarizes the dose-response pesticide activity results obtained with the seven blends. The second column with concentration zero (0) represent the pure petroleum jelly as negative control. Of the four dilutions of the blends tested, all the blends recorded 100% pesticide activity against the jigger flea, *Tunga penetrans*. It was clearly observed that nicotine is a very important component of the bioactive blend and its removal (blend ii) significantly lowers the insecticidal activity of the six components blend. The other five constituents of the blends are equally important in enhancing the pesticide activity of the blend components.

Table 2. Dose-response pesticide activity of the blend formulations against the jigger flea, *Tunga penetrans*

Blend	Pesticide activity at different concentrations ($\pm$ SE)				
	0	5	10	15	20
Blend (i)	0 ± 0.0^a	70.0 ± 2.0^b	100 ± 0.0^c	100 ± 0.0^c	100 ± 0.0^c
Blend (ii)	0 ± 0.0^a	25.0 ± 5.0^a	50 ± 2.5^a	100 ± 0.0^c	100 ± 0.0^c
Blend (iii)	0 ± 0.0^a	66.0 ± 2.5^b	100 ± 0.0	100 ± 0.0^c	100 ± 0.0^c
Blend (iv)	0 ± 0.0^a	60.0 ± 3.0^b	95 ± 2.5^b	100 ± 0.0^c	100 ± 0.0^c
Blend (v)	0 ± 0.0^a	65.0 ± 5.0^b	100 ± 0.0^c	100 ± 0.0^c	100 ± 0.0^c
Blend (vi)	0 ± 0.0^a	63.0 ± 3.0^b	100 ± 0.0^c	100 ± 0.0^c	100 ± 0.0^c
Blend (vii)	0 ± 0.0^a	63.5 ± 3.5^b	100 ± 0.0^c	100 ± 0.0^c	100 ± 0.0^c

Example 3

Pesticide activity of the 10% dilution of the six components synthetic blends on humans infested with the Jigger

The synthetic blend (i), comprising of a 10% formulation of six (6) constituents [Nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine in the ratio of 85: 2: 4: 7: 1: 1, respectively] and 90% petroleum jelly was tested for pesticide activity against the jigger flea, *Tunga penetrans* as a topical application on the humans infested by the flea (Figure 1). The weights of the compounds used in preparation of the 10% formulation were: Nicotine (85 wt%); nornicotine (2 wt%); Anabasine (4 wt%); Anatabine (7 wt%); Continine (1 wt%) and myosmine (1 wt%). The activity of the 10% formulation of the six components blend (blend i) was monitored for a week after the single topical application and photos on progress recorded. During the inspection, an infected part of the human body was considered jigger free if the

jiggers earlier present died. A live jigger usually oozes some liquid-like wastes at the tip of its tiny black spotted tail. Dryness at the tips of the jigger tail together with the embedded white body of the jigger changing to black followed by the dead jiggers falling off and shedding off of the dead human skin to normal skin was an considered as an indicator of the pesticide activity of the blend formulation as demonstrated in the images represented in the results for example 3. Application of the blend formulation is done on the foot of a jigger infested participant. Topical application of the blend formulation is done on the feet of the jigger infested participant.

10 Results.

The following figures of photo images of infected feet of some case studies involving some participants and taken before and one week after the topical application of the blend formulation clearly demonstrate the pesticide activity of the six components blend formulation on the jigger flea, *Tunga penetrans*.

15 Case Study 1

The invention has been able to demonstrates and carry out proof of the concept in the field on a number of male and female adult participants with both feet seriously infested with the flea before application of the blend formulation.

20 In some cases the right foot is heavily infested with jiggers and oozing liquid like wastes making it wet while in other cases the dorsal side of the right foot has deformed toes and oozing liquid wastes from the flea before application of the blend formulation as shown in **Fig. 2**. Some participants had the dorsal side of the right foot with many tiny white egg particles between the fingers.

25 In addition, the invention shows the results on the progress after topical application of the blend formulation (**Fig. 3**). **Fig. 3** also shows dead jiggers and the rejuvenation of the participant's normal skin on the feet. In some cases the invention shows the dorsal side of the right foot of a participant with all jiggers dead and falling off one week later as seen in **Fig. 3**. In the some cases, the ventral side of the foot of a participant with all jiggers dead and normal skin rejuvenating one week later. **Fig. 3** is able to show both feet of a participant with all jiggers' dead and normal skin rejuvenating one week later

Case Study 2

This involved a young adolescent youth whose both feet got infested by the jigger flea. Using soap, the feet were cleaned before application of the blend formulation. The feet with topical application of the formulation were left to heal and after one week it was revealed that the feet that are jigger free. Soap was preferred in cleaning jigger infested feet of participants before application of the blend formulation. Topical application of the blend formulation on jigger infested feet of participants was also preferred.

Best Way of Carrying out the Invention

In one of the best methods of executing the invention, the said bio-pesticide composition is demonstrated in Example 1 and case study 1 above.

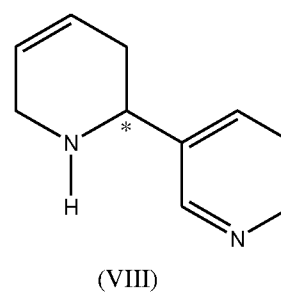
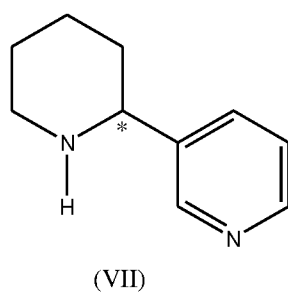
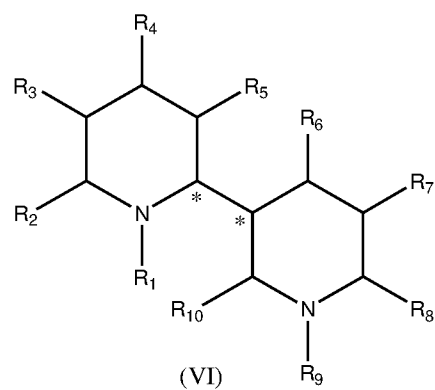
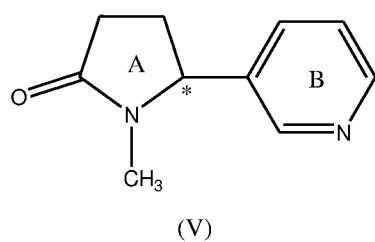
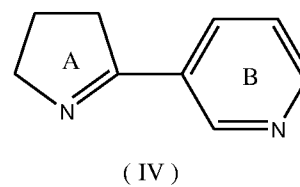
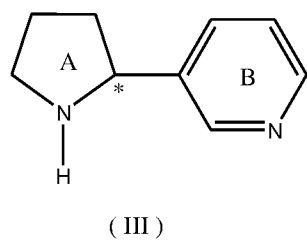
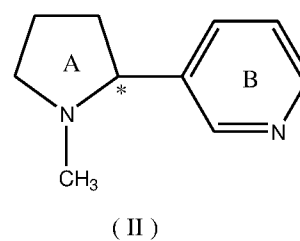
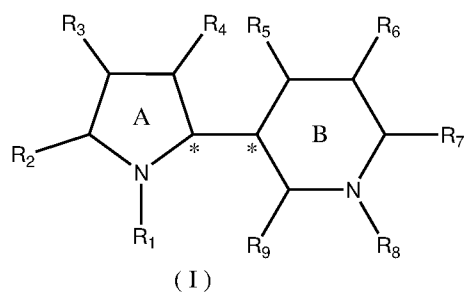
INDUSTRIAL APPLICATION

The present invention is applicable in chemical and agrochemical industry in controlling insect and arachnid pests among other arthropods. The composition is suitable for controlling the behaviour of insects or arachnids or arthropods, as well as methods of use of such compositions. The invention finds utility in the field of insect or arachnid or arthropod pest management for protection of human and animals against the pests of significant importance as far as human and animal diseases are concerned.

CLAIMS

What is claimed is:

1. A bio-pesticide composition for killing blood feeding insects and arachnid pests, comprising:
 - 5 a. a combination of nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine compounds from *Nicotiana tabacum* plant leaf,
 - b. a lemon *Eucalyptus* oil, and
 - c. petroleum jelly.
- 10 2. The bio-pesticide composition as in claim 1, wherein the seven synthetic blends further comprises 10% - 20% formulation of Nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine in the ratio of 85: 2: 4: 7: 1: 1 respectively in petroleum jelly base.
3. The bio-pesticide composition as in claim 1, wherein the petroleum jelly base is in the range of 80% - 90% and technically formulated to kill the jigger flea, *Tunga penetrans*.
- 15 4. The bio-pesticide composition as in claim 3, wherein the mode of application is topical on the surface of the victim's feet infested by the blood feeding parasites, *Tunga penetrans*.
5. The bio-pesticide composition as in claim 1, wherein the powdered dry leaves of about 50 gm is mixed with petroleum jelly of about 100 g and Lemon *Eucalyptus* oil of about
20 1 g and the resulting is mixed to make homogeneous solution.
6. The bio-pesticide composition as in claim 5, wherein the resultant formulation of the tobacco leaves is applied topically on the jigger infested parts on the body of the victim give 100% pesticide activity in a single application on a jigger infested victim within a period of 5-7 days.
- 25 7. A bio-pesticide composition for protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among others pests through the application of an effective amount of any one of the compounds having the structural formulae:



5 Where in (I), (II), (III), (IV) and (V), both rings A and B are either completely saturated in which case carbons marked with asterisk are chiral carbons (stereogenic centers) or completely unsaturated; or Ring A is completely saturated, in which case its asterisk carbon is chiral, while ring B is completely unsaturated; or Ring A is completely

unsaturated while ring B is completely saturated in which case its asterisk carbon is chiral; or Rings A and B are partially unsaturated or either A or B is partially or completely unsaturated; when ring A is partially or fully saturated and in which case R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ = H or an alkyl substituent as in nicotine (II),
5 nornicotine (III), myosine (IV) etc or R₂ = O atom doubly bonded to the carbon of ring A as in cotinine (V).

Where in (VI), (VII) and (VIII) both rings A and B are either completely saturated in which case carbons marked with asterisk are chiral carbons (stereogenic centers) or completely unsaturated; or Ring A is completely saturated, in which case its asterisk
10 carbon is chiral, while ring B is completely unsaturated; or Ring A is completely unsaturated while ring B is completely saturated in which case its asterisk carbon is chiral; or Rings A and B are partially unsaturated or either A or B is partially or completely unsaturated; when ring A is partially or fully saturated and in which case R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉ and R₁₀ = H or an alkyl substituent as in anabasine
15 (VII), anatabine (VIII)

8. The bio-pesticide composition for as in claim 7, wherein the application of effective amounts of blends further comprises any combinations of compounds having the above structural formulae for additive or augmentative effects.
9. The bio-pesticide composition for as in claim 7, wherein the said compound is nicotine
20 (II) [in which case R₁ = CH₃, R₂, R₃, R₄, R₅, R₆, R₇ and R₉ = H while R₈ has no atom i.e the N atom where R₈ is bonded is unsaturated]; or nornicotine (III) [in which case R₁, R₂, R₃, R₄, R₅, R₆, R₇ and R₉ = H while R₈ has no atom i.e the N atom where R₈ is bonded is unsaturated], or myosine (IV) [in which case R₂, R₃, R₄, R₅, R₆, R₇ and R₉ = H while R₁ and R₈ have no atom i.e the N atom where R₁ and R₈ are bonded are
25 unsaturated], or cotinine (V) [in which case R₁ = CH₃, R₂ = O, R₃, R₄, R₅, R₆, R₇ and R₉ = H while R₈ has no atom i.e the N atom where R₈ is bonded is unsaturated]; or anabasine (VII) [in which case R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, and R₁₀ = H while R₉ has no atom i.e the N atom where R₉ is bonded is unsaturated]; or anatabine (VIII) [in
30 which case R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, and R₁₀ = H while carbon atoms where R₃ and R₄ are bonded are SP² hybridized and R₉ has no atom i.e the N atom where R₉ is bonded is unsaturated].

10. The bio-pesticide composition for as in claim 9, wherein the said blends are made up of nicotine, normicotine, myosine, cotinine, anabasine and anatabine in any selected proportion.
- 5 11. The bio-pesticide composition for as in claim 7, 8, 9 or 10, wherein the said compounds or blends are applied with other deterrents or repellents or insecticides or acaricides, synthetic or naturally occurring, in any selected proportion for augmentative effects.
- 10 12. The bio-pesticide composition for as in claim 7, 8, 9, 10 or 11, wherein the said compound(s) is/are applied in a suitable carrier, diluent, additives such as antioxidants, oxidizing agents, antimicrobials, acids, bases, buffers, colorants, crystal growth inhibitors, de-foaming agents, emollients, fillers, gelling agents, fragrances, lubricants, propellants, thickeners, salts, solvents, surfactants, UV-screens, other chemical stabilizers, hydrocarbon constituents and mixtures thereof.
- 15 13. The bio-pesticide composition for as in claim 7, 8, 9, 10, 11 or 12, wherein the compound(s) is/are contained in suitable formulations or devices for convenience of handling and/or controlled release for extending period of effective performance.
- 20 14. A bio-pesticide composition for masking the activity of insect attractant compounds, the said composition, comprising applying a compound selected from: compounds having the structural formulae; (I) or (II) or (III) or (IV) or (V) or (VI) or (VII) or (VIII) as defined in claim 7.
- 25 15. The bio-pesticide composition as described in claims 7 to 14, wherein the insect or arachnid (or arthropod) is another blood feeding insect pest or arachnid pest species such as the mosquitoes, tsetse flies, black flies, sand flies, muscids, tabanids, tachanids, bedbugs, ticks, mites, etc.
16. The bio-pesticide composition as in claim 15, wherein the compound is in a composition that further comprises one or more additives.
17. The bio-pesticide composition as in claim 16, wherein the compound is applied on or near an animal host and wherein the insect attractant compounds are produced by, or present on, the animal host.
- 30 18. The bio-pesticide composition as in claim 17, wherein the host is a human or other animals, and wherein the compound is effective to reduce insect or arachnid or arthropod nuisance to the human or other animals.

19. The bio-pesticide composition as claim 18, wherein the compound is in a liquid composition further comprising a carrier, and wherein the compound is applied by releasing the composition into an environment containing insects or arachnids.
- 5 20. A method of protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among other blood feeding insects and arachnids pests by repelling or killing the insects or arachnids through periodic or continuous controlled-release space fumigation with compounds of structural formulae (I) or (II) or (III) or (IV) or (V) or (VI) or (VII) or (VIII) as defined in claim 7.
- 10 21. The method of protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among other blood feeding insects and arachnid pests as in claim 14, wherein two or more of the compounds are used in blends for additive or augmentative effects.
- 15 22. The method of protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among other blood feeding insects and arachnids pests as in claim 14, wherein the said compound is nicotine (II) [in which case $R_1 = CH_3$, R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and $R_9 = H$ while R_8 has no atom i.e the N atom where R_8 is bonded is unsaturated]; or nornicotine (III) [in which case R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and $R_9 = H$ while R_8 has no atom i.e the N atom where R_8 is bonded is unsaturated], or myosine (IV) [in which case R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and $R_9 = H$ while R_1 and R_8 have no atom i.e the N atom where R_1 and R_8 are bonded are unsaturated], or cotinine (V) [in which case $R_1 = CH_3$, $R_2 = O$, R_3 , R_4 , R_5 , R_6 , R_7 and $R_9 = H$ while R_8 has no atom i.e the N atom where R_8 is bonded is unsaturated]; or anabasine (VII) [in which case R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , and $R_{10} = H$ while R_9 has no atom i.e the N atom where R_9 is bonded is unsaturated]; or anatabine (VIII) [in which case R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_8 , and $R_{10} = H$ while carbon atoms where R_3 and R_4 are bonded are SP^2 hybridized and R_9 has no atom i.e the N atom where R_9 is bonded is unsaturated].
- 25 23. The method of protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among other blood feeding insects and arachnid pests as in claim 15, wherein the said blends are made up of nicotine, nornicotine, myosine, cotinine, anabasine and anatabine in any selected proportion.
- 30 24. The method of protecting humans and other animals from blood feeding insects and arachnids as in claims 14, 15, 16, or 17, wherein the said compound(s) is/are used with

other repellents, synthetic or naturally occurring, for augmentative effects in any selected proportion.

25. The method of protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among other blood feeding insects and arachnid pests as described in claims 14, 15, 16, 17, or 18, wherein the said compound(s) or blend(s) is/are released in a suitable carrier or diluent with/without additives referred to in claim 12.

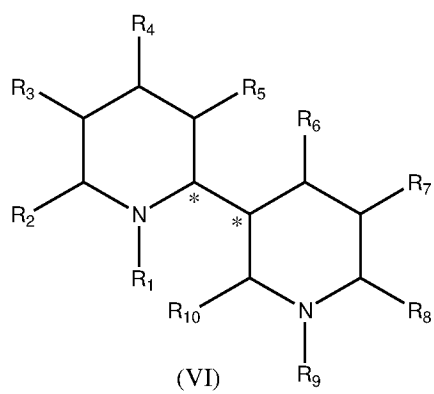
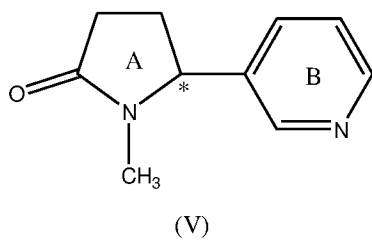
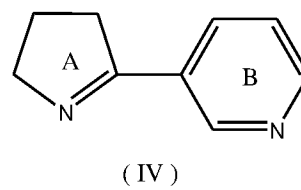
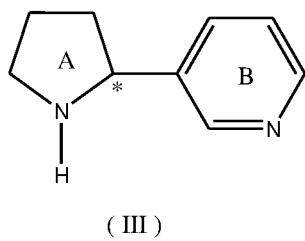
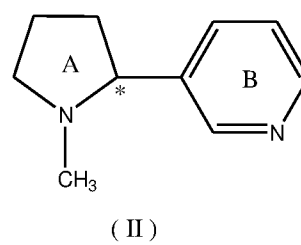
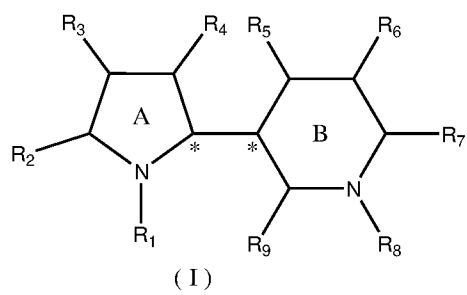
26. A method of formulating a bio-pesticide composition consisting of Nicotine and other bio-molecules consisting of steps of:

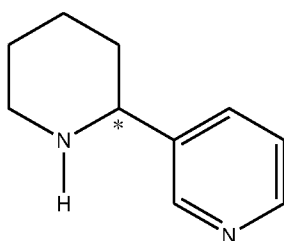
- a. Drying and grounding leaves of *Nicotiana tabacum* plant leaf in water,
- b. Extraction of nicotine, nornicotine, Anabesine, Anatabine, Continine and myosmine compounds using sequential solvent method using hexane – methyl – ethyl acetate
- c. mixing of the above compound with lemon *Eucalyptus* oil and petroleum jelly as a base
- d. Melting the jelly at around 45⁰C and allowing it to settle for about 12 hours,
- e. Re-mixing the solution by stirring the composition to form a homogeneous solution,
- f. Applying to the victim at least thrice a day for 5 – 7 days.

AMENDED CLAIMS
received by the International Bureau on 20 November 2021 (20.11.21)

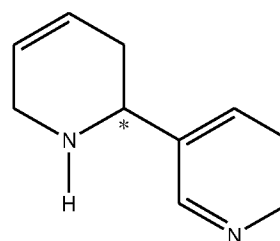
5 What is claimed is:

1. A bio-pesticide composition for killing blood feeding insects and arachnid pests, comprising:
 - a combination of nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine compounds from *Nicotiana tobacum* plant leaf,
 - 10 a lemon *Eucalyptus* oil, and
 - petroleum jelly.
2. The bio-pesticide composition as in claim 1, wherein the seven synthetic blends further comprises 10% - 20% formulation of Nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine in the ratio of 85: 2: 4: 7: 1: 1 respectively in petroleum jelly
- 15 base.
3. The bio-pesticide composition as in claim 1, wherein the petroleum jelly base is in the range of 80% - 90% and technically formulated to kill the jigger flea, *Tunga penetrans*.
4. The bio-pesticide composition as in claim 3, wherein the mode of application is topical on the surface of the victim's feet infested by the blood feeding parasites, *Tunga penetrans*.
- 20
5. The bio-pesticide composition as in claim 1, wherein the powdered dry leaves of about 50 gm is mixed with petroleum jelly of about 100 g and Lemon *Eucalyptus* oil of about 1 g and the resulting is mixed to make homogeneous solution.
6. (Amended) The bio-pesticide composition as in claim 5, wherein the resultant
- 25 formulation of the tobacco leaves is applied topically on the jigger infested parts on the body of the victim to give 100% pesticide activity in a single application on a jigger infested victim within a period of 5-7 days, wherein:
 - the resultant formulation further comprises an effective amount of any one of the compounds having the structural formulae:





(VII)



(VIII)

wherein (I), (II), (III), (IV) and (V), both rings A and B are either completely saturated in which case carbons marked with asterisk are chiral carbons (stereogenic centers) or completely unsaturated; or Ring A is completely saturated, in which case its asterisk carbon is chiral, while ring B is completely unsaturated; or Ring A is completely unsaturated while ring B is completely saturated in which case its asterisk carbon is chiral; or Rings A and B are partially unsaturated or either A or B is partially or completely unsaturated; when ring A is partially or fully saturated and in which case R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ = H or an alkyl substituent as in nicotine (II), nornicotine (III), myosine (IV) etc or R₂ = O atom doubly bonded to the carbon of ring A as in cotinine (V);

wherein (VI), (VII) and (VIII) both rings A and B are either completely saturated in which case carbons marked with asterisk are chiral carbons (stereogenic centers) or completely unsaturated; or Ring A is completely saturated, in which case its asterisk carbon is chiral, while ring B is completely unsaturated; or Ring A is completely unsaturated while ring B is completely saturated in which case its asterisk carbon is chiral; or Rings A and B are partially unsaturated or either A or B is partially or completely unsaturated; when ring A is partially or fully saturated and in which case R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉ and R₁₀ = H or an alkyl substituent as in anabasine (VII), anatabine (VIII);

the application of effective amounts of blends further comprises any combinations of compounds having the above structural formulae for additive or augmentative effects;

the said compound is nicotine (II) [in which case R₁ = CH₃, R₂, R₃, R₄, R₅, R₆, R₇ and R₉ = H while R₈ has no atom i.e the N atom where R₈ is bonded is unsaturated]; or nornicotine (III) [in which case R₁, R₂, R₃, R₄, R₅, R₆, R₇ and R₉ = H while R₈ has no atom i.e the N atom where R₈ is bonded is unsaturated], or

myosine (IV) [in which case R2, R3, R4, R5, R6, R7 and R9 = H while R1 and R8 have no atom i.e the N atom where R1 and R8 are bonded are unsaturated], or cotinine (V) [in which case R1 = CH₃, R2 = O, R3, R4, R5, R6, R7 and R9 = H while R8 has no atom i.e the N atom where R8 is bonded is unsaturated]; or anabasine (VII) [in which case R1, R2, R3, R4, R5, R6, R7, R8, and R10 = H while R9 has no atom i.e the N atom where R9 is bonded is unsaturated]; or anatabine (VIII) [in which case R1, R2, R3, R4, R5, R6, R7, R8, and R10 = H while carbon atoms where R3 and R4 are bonded are SP² hybridized and R9 has no atom i.e the N atom where R9 is bonded is unsaturated], and

the said blends are made up of nicotine, nornicotine, myosine, cotinine, anabasine and anatabine in any selected proportion.

7. Cancelled

8. Cancelled

9. Cancelled

10. Cancelled

11. (Amended) The bio-pesticide composition as in claim 6, wherein the said compounds or blends are applied with other deterrents or repellents or insecticides or acaricides, synthetic or naturally occurring, in any selected proportion for augmentative effects.

12. (Amended) The bio-pesticide composition as in claim 11, wherein the said compound(s) is/are applied in a suitable carrier, diluent, additives such as antioxidants, oxidizing agents, antimicrobials, acids, bases, buffers, colorants, crystal growth inhibitors, de-foaming agents, emollients, fillers, gelling agents, fragrances, lubricants, propellants, thickeners, salts, solvents, surfactants, UV-screens, other chemical stabilizers, hydrocarbon constituents and mixtures thereof.

13. (Amended) The bio-pesticide composition as in claim 12, wherein the compound(s) is/are contained in suitable formulations or devices for convenience of handling and/or controlled release for extending period of effective performance, wherein:

the said bio-pesticide composition is applied to mask the activity of insect attractant compounds, the said composition, comprising applying a compound selected from: compounds having the structural formulae; (I) or (II) or (III) or (IV) or (V) or (VI) or (VII) or (VIII) as defined in claim 6;

the insect or arachnid (or arthropod) is another blood feeding insect pest or arachnid pest species such as the mosquitoes, tsetse flies, black flies, sand flies, muscids, tabanids, tachanids, bedbugs, ticks and mites;

the compound is in a composition that further comprises one or more additives;

5 the compound is applied on or near an animal host and wherein the insect attractant compounds are produced by, or present on, the animal host, and

the host is a human or other animals, and wherein the compound is effective to reduce insect or arachnid or arthropod nuisance to the human or other animals.

14. Cancelled.

10 15. Cancelled.

16. Cancelled.

17. Cancelled.

18. Cancelled.

15 19. (Amended) The bio-pesticide composition as in claim 13, wherein the compound in a liquid composition further comprising a carrier, and wherein the compound is applied by releasing the composition into an environment containing insects or arachnids.

20 20. (Amended) A method of protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among other blood feeding insects and arachnids pests by repelling or killing the insects or arachnids through periodic or continuous controlled-release space fumigation with compounds of structural formulae (I) or (II) or (III) or (IV) or (V) or (VI) or (VII) or (VIII) as defined in claim 6.

25 21. (Amended) The method of protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among other blood feeding insects and arachnid pests as in claim 13, wherein two or more of the compounds are used in blends for additive or augmentative effects.

30 22. (Amended) The method of protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among other blood feeding insects and arachnids pests as in claim 13, wherein the said compound is nicotine (II) [in which case $R_1 = \text{CH}_3$, $R_2, R_3, R_4, R_5, R_6, R_7$ and $R_9 = \text{H}$ while R_8 has no

atom i.e the N atom where R₈ is bonded is unsaturated]; or nornicotine (III) [in which case R₁, R₂, R₃, R₄, R₅, R₆, R₇ and R₉ = H while R₈ has no atom i.e the N atom where R₈ is bonded is unsaturated], or myosine (IV) [in which case R₂, R₃, R₄, R₅, R₆, R₇ and R₉ = H while R₁ and R₈ have no atom i.e the N atom where R₁ and R₈ are bonded are unsaturated], or cotinine (V) [in which case R₁ = CH₃, R₂ = O, R₃, R₄, R₅, R₆, R₇ and R₉ = H while R₈ has no atom i.e the N atom where R₈ is bonded is unsaturated]; or anabasine (VII) [in which case R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, and R₁₀ = H while R₉ has no atom i.e the N atom where R₉ is bonded is unsaturated]; or anatabine (VIII) [in which case R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, and R₁₀ = H while carbon atoms where R₃ and R₄ are bonded are SP² hybridized and R₉ has no atom i.e. the N atom where R₉ is bonded is unsaturated].

23. (Amended) The method of protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among other blood feeding insects and arachnid pests as in claim 15, wherein the said blends are made up of nicotine, nornicotine, myosine, cotinine, anabasine and anatabine in any selected proportion.

24. (Amended) The method of protecting humans and other animals from blood feeding insects and arachnids as in claim 13, wherein the said compound(s) is/are used with other repellents, synthetic or naturally occurring, for augmentative effects in any selected proportion.

25. The method of protecting humans and other animals from blood feeding insects and arachnids such as the jigger flea, *Tunga penetrans* among other blood feeding insects and arachnid pests, wherein the said compound(s) or blend(s) is/are released in a suitable carrier or diluent with/without additives referred to in claim 13.

26. A method of formulating a bio-pesticide composition consisting of Nicotine and other bio-molecules consisting of steps of:

- a. Drying and grounding leaves of *Nicotiana tabacum* plant leaf in water,
- b. Extraction of nicotine, nornicotine, Anabasine, Anatabine, Continine and myosmine compounds using sequential solvent method using hexane – methyl – ethyl acetate
- c. mixing of the above compound with lemon *Eucalyptus* oil and petroleum jelly as a base

- d. Melting the jelly at around 45⁰C and allowing it to settle for about 12 hours,
- e. Re-mixing the solution by stirring the composition to form a homogeneous solution,
- f. Applying to the victim at least thrice a day for 5 – 7 days.

STATEMENT UNDER ARTICLE 19 (1)

5 This Statement concerns the above international patent application for an invention entitled “**Bio-Pesticide Composition for Killing Jiggers and other Blood Feeding Insects and Arachnid Pests.**” The said Statement is generated under Article 19(1) of Patent Cooperation Treaty (PCT) and seeks to explain the reasons underlying amendments to the presented claims as for entering national phase of PCT as follows:

10

1. Claims 1-5 are retained as originally filed.
2. Claim 6 has been amended to incorporate important novel and inventive features of claims 7-10 as originally dependent. The basis for this amendment is based on the fact that whereas the prior art D1 relates to a kind of nicotine found in 11 kinds of secondary alkaloid impurity contents method of physico-chemical analysis and D3 relates to a skin coating compositions and methods containing an active agent, the present invention teaches of a bio-pesticide composition for killing jiggers and other blood feeding insects and arachnid pests which has more than just the disclosed nicotine and related secondary alkaloids which could not have been easily anticipated by the persons skilled in the art.
- 15 3. Claims 7-10 are cancelled since they had been anticipated by prior art document D1 & D2
4. Claims 11-13 are amended as dependent of the amended claim 6, 11 and 12 respectively.
5. Claim 13 has been amended to incorporate important novel and inventive features of claims 14-18. The basis for this amendment is based on the fact that whereas the prior art D1 relates to a kind of nicotine found in 11 kinds of secondary alkaloid impurity contents method of physico-chemical analysis and D3 relates to a skin coating compositions and methods containing an active agent, the present invention teaches of a bio-pesticide composition for killing jiggers and other blood feeding insects and arachnid pests which
- 25

has more than just the disclosed nicotine and related secondary alkaloids which could not have been easily anticipated by the persons skilled in the art.

6. Claims 14-18 have been cancelled since they had been anticipated in prior art documents D1 & D2.

5 7. Claim 19 has been amended to incorporate important novel and inventive features of claims 14-18. The basis for this amendment is based on the fact that whereas the prior art D1 relates to a kind of nicotine found in 11 kinds of secondary alkaloid impurity contents method of physico-chemical analysis and D3 relates to a skin coating compositions and methods containing an active agent, the present invention teaches of a bio-pesticide
10 composition for killing jiggers and other blood feeding insects and arachnid pests which has more than just the disclosed nicotine and related secondary alkaloids which could not have been easily anticipated by the persons skilled in the art.

8. Claims 20-25 are amended as dependent of the amended claim 13 and 15 accordingly.

9. Claim 26 has been retained as filed since it had not been anticipated in any of the cited
15 prior art documents D1, D2 or D3.

FIGURES

Fig. 1/4: Normal Feet

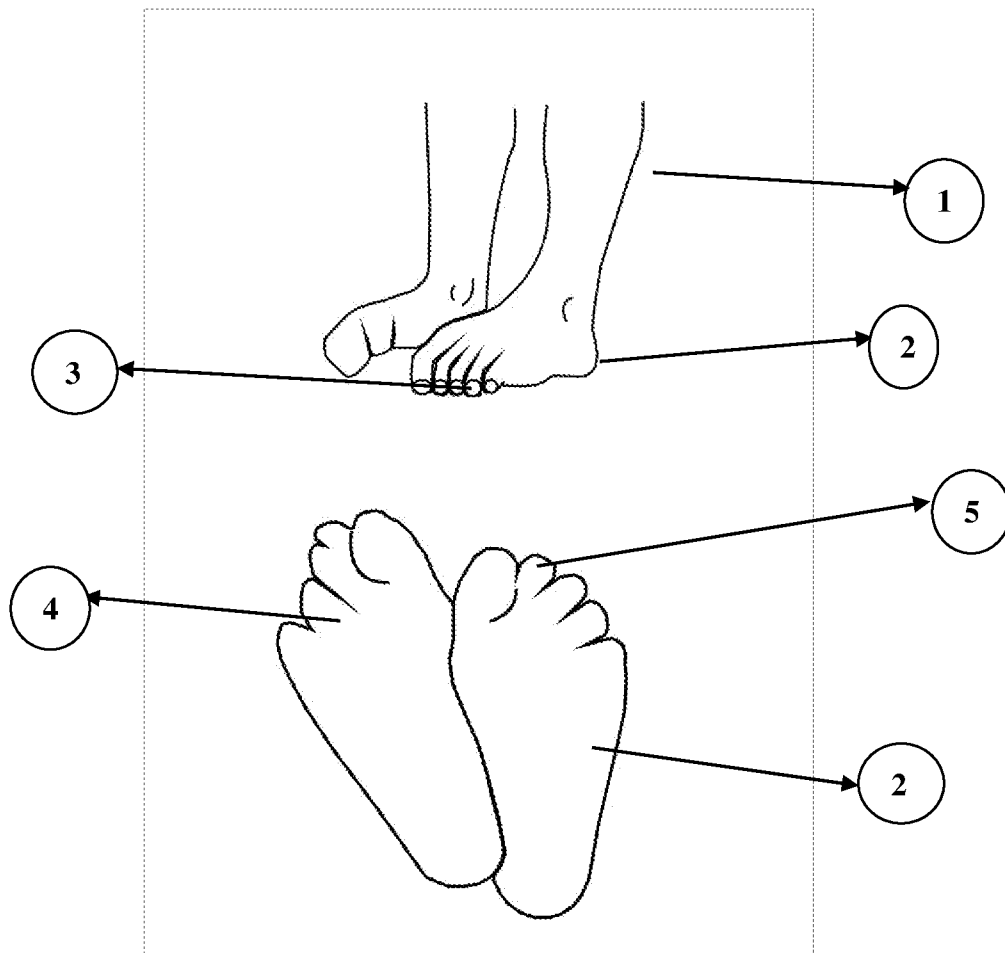


Fig. 2/4: Jigger Infested Feet

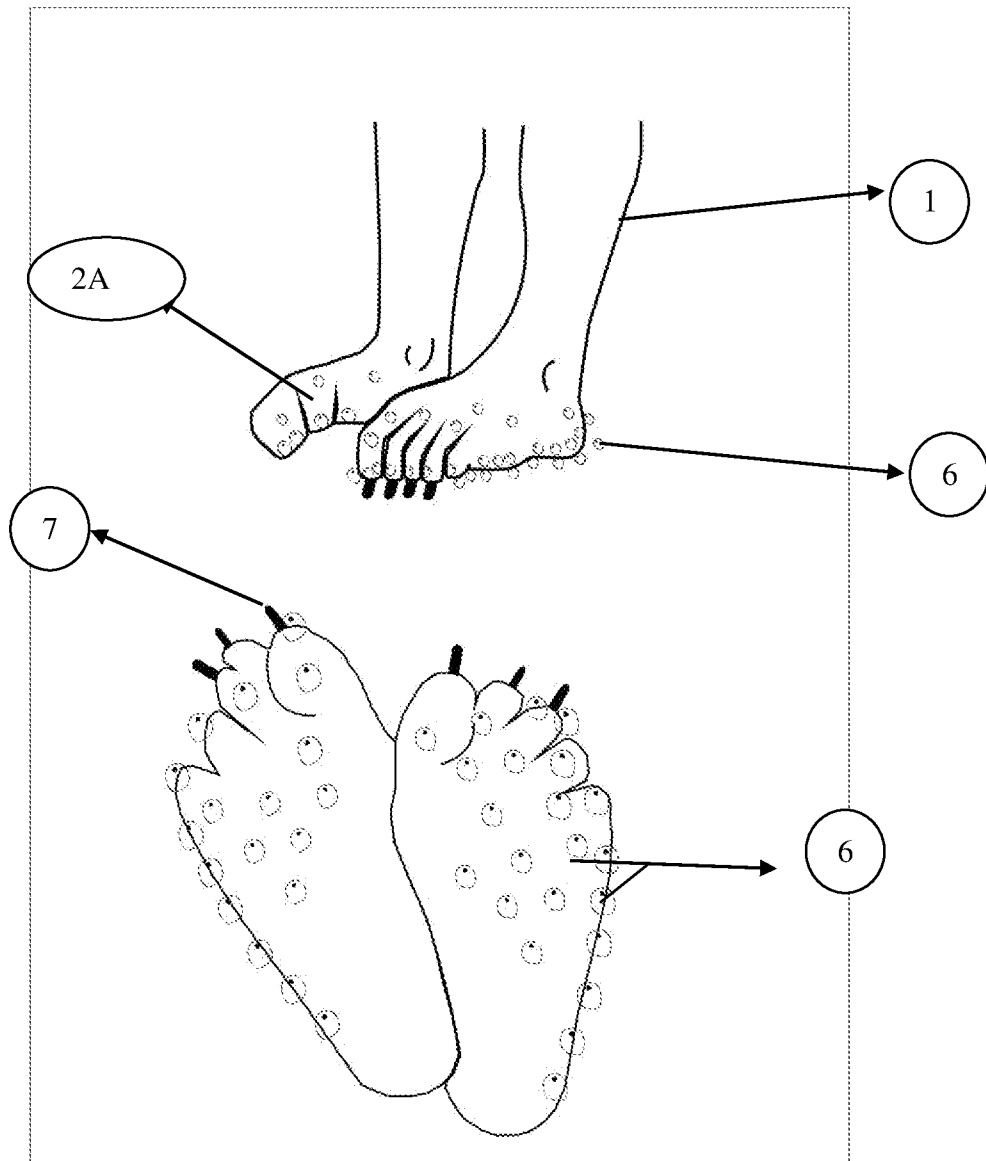


Fig. 3/4: After Jigger Infestation Treatment

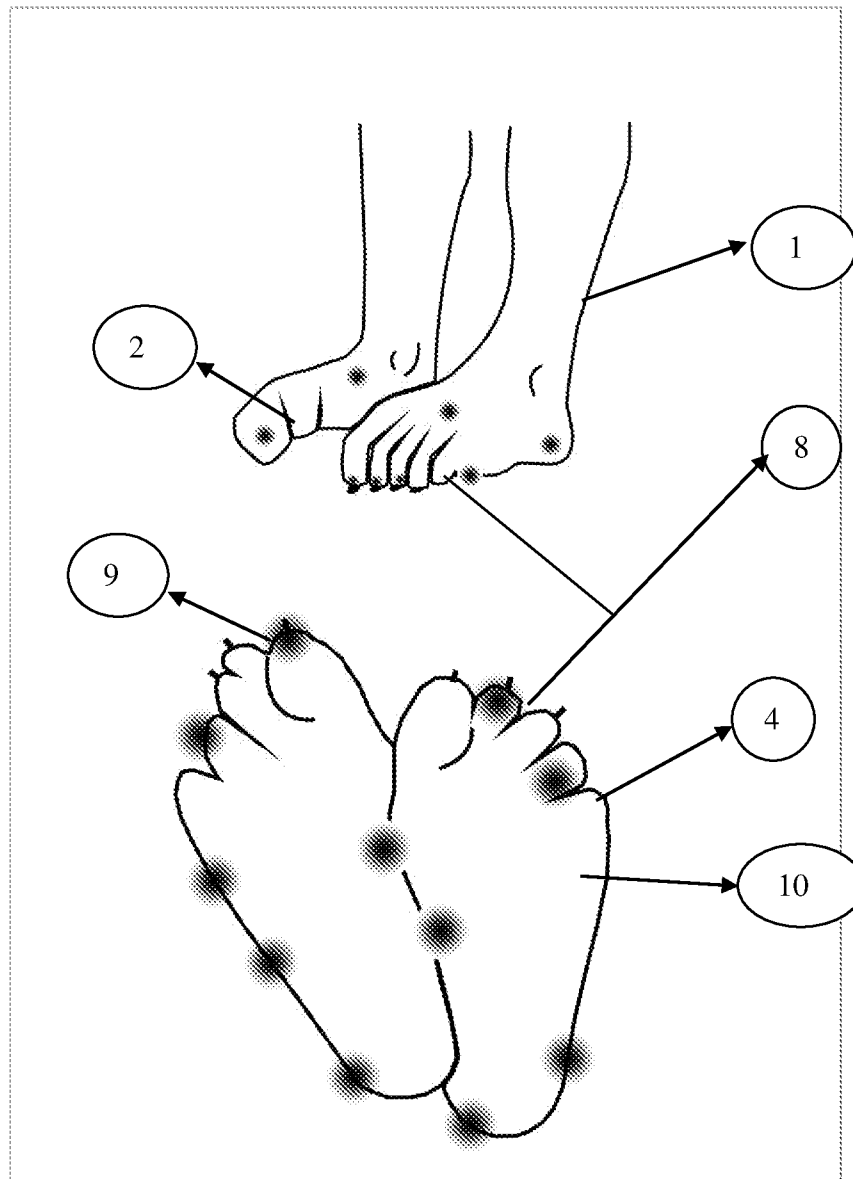
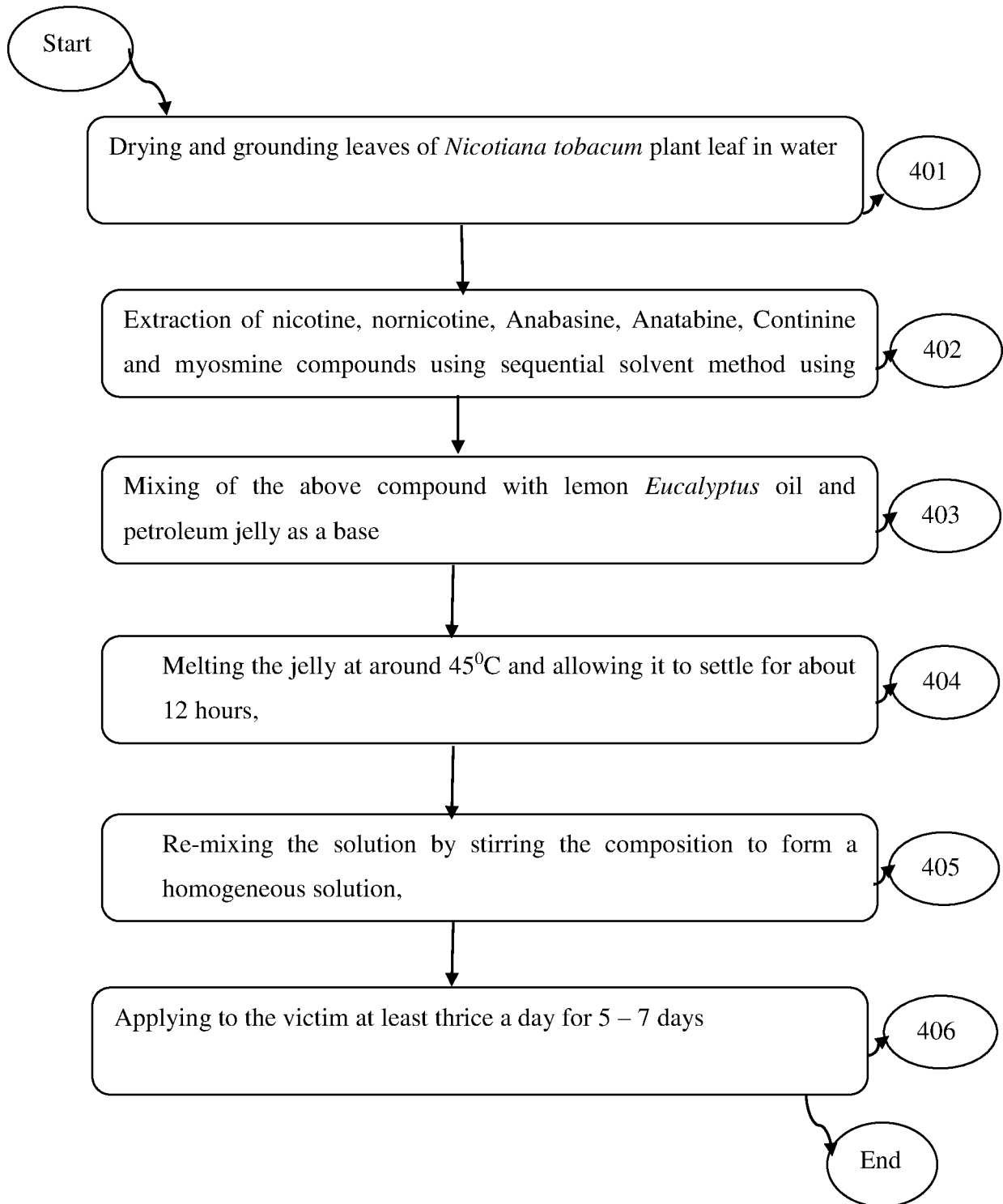


Fig. 4/4: Schematic Flow Chart for Method of Formulating Anti-tungiasis



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KE2021/050001

A. CLASSIFICATION OF SUBJECT MATTER

A01P 7/04(2006.01)i; A01P 7/02(2006.01)i; A01N 25/04(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A01P7/-; A01N25/-

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, CNABS, CNKI, REGISTRY(STN), CAPLUS(STN):nicotine, nornicotine, anabasine, anatabine, continine, myosmine,pesticide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 107255687 A (NAT TOBACCO QUALITY SUPERVISION & INSPEC) 17 October 2017 (2017-10-17) see paragraphs [0002], [0005]; Figure 2	7-19
X	MING, Ningning et al. "Extraction and analysis of alkaloids in tobacco:a review" <i>Chinese Journal of tobacco</i> , Vol. 13, No. 3, 30 June 2007 (2007-06-30), pages 64-70	7-19
A	WO 2011038120 A1 (PHARMASOL CORPORATION) 31 March 2011 (2011-03-31) see claims 28, 32	1-26
A	CN 106305722 A (LIU, Yisu) 11 January 2017 (2017-01-11) see paragraphs [0001]-[0002], claim 1	1-26



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 September 2021

Date of mailing of the international search report

18 October 2021

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/KE2021/050001

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	107255687	A	17 October 2017	None			
WO	2011038120	A1	31 March 2011	MX	2012003588	A	25 July 2012
				BR	112012006756	A2	17 May 2016
				CN	102665734	A	12 September 2012
				EP	2480238	A1	01 August 2012
				US	2011076244	A1	31 March 2011
CN	106305722	A	11 January 2017	None			