

Supplemental file 2

Supplemental file 2.1: Fortaleza classification system

Embedded sand flea lesions are stratified into different developmental stages, as per the Fortaleza classification system¹.

Table 1: Fortaleza classification system

Stages	Appearance/phases	Symptoms	Time span
Stage I	Penetrating flea (penetration)	Erythema, and itching	30 min–several hours
Stage II	Brownish-black dot (beginning of hypertrophy)	Erythema surrounding a central black dot, unpleasant itching, and pain	1–2 days after penetration
Stage III _a	White (tender) halo with black dot at the centre (hypertrophy)	Eggs expulsion, faecal coil, brownish-watery secretion, pulsation, severe itching, pain, and tenderness	2–6 days after penetration
Stage III _b	White (non-tender) halo with caldera formation, discoloration, and skin peeling around lesion (hypertrophy)	Eggs (white and shining) expulsion, faecal coil, pulsation, watery secretion, severe pain while walking, and loss of tenderness	6 days–3 weeks after penetration
Stage IV _a	Brownish-black wrinkled lesion (involution)	Rare egg expulsion and pulsation, sporadic faecal expulsion, and watery secretion	3–4 weeks after penetration
Stage IV _b	Brownish-black, necrotised, desiccated lesion (crust) (involution)	No vital signs (pulsation, egg, faeces, and watery secretion), (dead flea)	4–6 weeks after penetration
Stage V	Circular depression in the stratum corneum (residue)	No flea	6 weeks–several months after penetration

Stage II and III lesions can be classified as viable embedded sand flea lesions, whereas stage IV is classified as a lesion with either a dying (IV_a) or dead (IV_b) embedded flea. An embedded sand flea is considered to be viable when any of the viability signs (expulsion of eggs, excretion of faecal threads, excretion of faecal liquid, and/or pulsations/contractions) are observed using diagnostic tools (hand held digital microscope).¹

Supplemental file 2.2: Study schedule

Table 2: Study schedule of enrolment, interventions, and assessments.

Study procedures	Time points					
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
	Day 0*	Day 1	Day 4	Day 5	Day 7	Day 10
Recruitment and enrolment						
Training clinical recruitment and study team	X					
Identifying potential participants with tungiasis	X					
Participant information sheet	X					
Informed consent/assent	X					
Subject demographics / medical history	X					
Inclusion/exclusion criteria - review	X					
Concomitant medications - review	X					

Subjects instructions	X					
Subject randomisation	X					
Baseline assessment-lesion viability & staging	X					
Baseline assessment-acute tungiasis morbidity	X					
Study intervention						
Distribution of intervention products	X	X	X		X	
Application of test intervention		X	X		X	
Application of control intervention		X	X		X	
Outcome assessment						
Efficacy outcome-viability of embedded sand flea				X		X
Acute morbidity outcome-SSAT, itching & sleep disturbance				X		X
Safety outcome-monitoring AEs		X	X	X	X	X
Product acceptability outcome				X		X
Study compliance confirmation		X	X	X	X	X

Supplemental file 2.3: Adverse events grading

Table 3: Grading severity of adverse events.

Grade	Type	Description
Grade 1	Mild	Signs or symptoms which are easily tolerated, does not interfere with the subject's usual function; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate	Signs or symptoms causes interference with usual activity or affects clinical status; minimal, local or non-invasive intervention indicated
Grade 3	Severe	Signs or symptoms affect clinical status and likely requires medical intervention and/or close follow-up
Grade 4	Life-threatening	Sign or symptom results in a potential threat to life; urgent intervention indicated This grade will be considered as SAE

Supplemental file 2.4: Parasiticidal and repellent effects of tea tree oil (TTO)

Table 4: Summary of studies on the insecticidal, acaricidal, and repellent effects of TTO.

Study setting	Study design	TTO concentration or volume tested	Ectoparasite (insect or arachnid)	Treatment outcome
Akkad <i>et al.</i> 2016, ² Egypt	<i>In vitro</i>	5% TTO Head Lice Gel	Louse (<i>Pediculus humanus capitis</i>)	96.7% mortality
Alver <i>et al.</i> 2017, ³ Turkey	<i>In vivo</i>	10% TTO eye shampoo with 4% gel	Mite (<i>Demodex folliculorum</i> & <i>D. brevis</i>)	82.1% improvement in blepharitis
Barker & Altman 2010, ⁴ Australia	RCT	10% w/v TTO and 1% w/v lavender oil NeutraLice Lotion® (TTO/LO)	Louse (<i>Pediculus humanus capitis</i>)	97.6% cure rate
Benelli <i>et al.</i> 2013, ⁵ Italy	<i>In vitro</i>	1.5-3 µL oil/cm ² TTO	Mediterranean fruit fly (<i>Ceratitis capitata</i>)	>60% mortality
Callander & James 2012, ⁶ Australia	<i>In vitro</i>	2.5-3% TTO	Blow fly (<i>Lucilia cuprina</i>)	100 % ovicidal and larvicidal (1st instar) & 100% repellent effect for 7hrs
De Wolff 2008, ⁷ USA	<i>In vitro</i>	20% TTO	Fleas (<i>Siphonaptera</i>)	78% mortality(in 1hr) and

				100% mortality (in day)
Di Campli <i>et al.</i> 2012, ⁸ Italy	<i>In vitro</i>	1-8 % TTO	Louse (<i>Pediculus humanus capitis</i>)	100 % mortality
Ellse <i>et al.</i> 2013, ⁹ UK	<i>In vitro</i> <i>In vivo</i>	5% & 10% TTO 5% TTO	Donkey chewing louse (<i>Bovicola (Werneckiella) Ocellatus</i>)	>80% mortality
Ellse <i>et al.</i> 2016, ¹⁰ UK	<i>In vivo</i>	5% TTO	Donkey chewing louse (<i>Bovicola (Werneckiella) Ocellatus</i>)	78% mortality
Fitzjarrell 1995, ¹¹ USA	<i>In vivo</i>	2–10% v/v TTO	Fleas (<i>Siphonaptera</i>)	100% mortality
Gao <i>et al.</i> 2005, ¹² USA	<i>In vitro</i> and <i>in vivo</i>	50–100% TTO	Mite (<i>Demodex folliculorum</i>)	100% mortality
Iori <i>et al.</i> 2005, ¹³ Italy	<i>In vitro</i>	8 -10µl TTO	Tick (<i>Ixodes ricinus</i>)	>80% mortality
James & Callander 2012, ¹⁴ Australia	<i>In vitro</i>	1–20% TTO	Sheep louse (<i>Bovicola ovis Schrank</i>)	100% mortality (adult lice and eggs)
James & Callander 2012, ¹⁵ Australia	<i>In vivo</i>	1–2% TTO	Sheep louse (<i>Bovicola ovis Schrank</i>)	100% mortality
Klauck <i>et al.</i> 2014, ¹⁶ Brazil.	<i>In vitro</i>	5.0% TTO	Houseflies (<i>Musca domestica</i> & <i>H. irritans</i>)	100% mortality
Maher 2018, ¹⁷ United Arab Emirates	<i>In vivo</i>	5% TTO eyelid scrub	Mite (<i>Demodex folliculorum</i>)	100% improvement in symptoms
Nicholls <i>et al.</i> 2016, ¹⁸ Australia	<i>Case series (in vivo)</i>	5 % TTO	Mites (<i>Demodex folliculorum</i> & <i>D. brevis</i>)	91% improvement in symptoms
Pazinato <i>et al.</i> 2014, ¹⁹ Brazil	<i>In vitro</i>	1–10 % TTO & 0.075–0.75 % TTO nanoparticles	Tick (<i>Rhipicephalus (Boophilus) microplus</i>)	100 % reproductive inhibition 70 % mortality
Sands <i>et al.</i> 2016, ²⁰ UK	<i>In vitro</i>	5% TTO	Donkey chewing louse (<i>Bovicola (Werneckiella) Ocellatus</i>)	100% mortality
Talbert & Wall 2012, ²¹ UK	<i>In vitro</i>	0.5–10% TTO	Donkey chewing louse (<i>Bovicola (Werneckiella) Ocellatus</i>)	100% mortality
Walton <i>et al.</i> 2004, ²² Australia	<i>In vitro</i>	5% TTO	Scabies mite (<i>S scabiei var hominis</i>)	100% mortality
Walton <i>et al.</i> 2000, ²³ Australia	<i>in vitro</i>	5% TTO	Scabies mite (<i>S scabiei var hominis</i>)	100% mortality
Williamson <i>et al.</i> 2007, ²⁴ UK	<i>In vitro</i>	10% TTO	House dust mites (<i>Dermatophagoides pteronyssinus</i> & <i>D. farinae</i>); Louse (<i>Pediculus humanus capititis</i>)	100% immobility 100% mortality
Yim <i>et al.</i> 2016, ²⁵ Australia	<i>In vivo</i>	2–5% TTO	Cattle tick (<i>Rhipicephalus australis</i>)	78–100% repellent effect for 2 days

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