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# ENTOMOLOGY

# Knock down and insecticidal activity of the plants *Tagetes minuta*, *Lippia javanica*, *Lantana camara*, *Tagetes erecta* and *Eucalyptus grandis* on *Anopheles arabiensis* mosquitoes

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# Abstract

The knock down and insecticidal effects of the plants *Tagetes minuta*, *Lippia javanica*, *Lantana camara*, *Tagetes erecta* and *Eucalyptus grandis* were evaluated against *Anopheles arabiensis* mosquitoes in thatched round huts in Mumurwi village. Leaves from these plants were smouldered in order to provide mosquito

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repellent smoke. Complete knock down was provided 40 minutes after mosquitoes were exposed to smoke of T. erecta, 60 minutes to smoke of T. minuta and E. grandis and 120 minutes to smoke of L. javanica. Complete knock down of mosquitoes could not be provided by L. camara within the 140-minute exposure period. The KT<sub>50</sub> (time required to knock down 50% of the mosquitoes) values were 24.985 minutes (T. minuta), 34.473 minutes (T. erecta), 59.119 minutes (L. javanica), 59.828 minutes (L. camara) and 25.245 minutes (E. grandis). The KT<sub>90</sub> (time required to knock down 90% of the mosquitoes) values were 48.060 minutes (T. minuta), 50.169 minutes (T. erecta), 178.341 minutes (L. javanica), 140.220 minutes (L. camara) and 47.998 minutes (E. grandis). Mortality rates 24h after exposure were 40% (T. minuta), 100% (T. erecta), 75% (L. javanica), 90% (L. camara) and 100% (E. grandis). In conclusion, smoke from the plants T. erecta, T. minuta and E. grandis had very fast knock down rates with T. erecta, L. camara and E. grandis killing over 90% of the An. arabiensis mosquitoes. Plant smoke is important in mosquito control.

# Introduction

Mosquitoes have been associated with painful bites, nuisance and disease transmission in many parts of the world. Some mosquitoes are vectors of malaria, filariasis and arboviruses (dengue, West Nile, Zika) and females from some mosquito species bite both humans and animals (Pavela & Benelli, 2016). Prevention of mosquito bites is the key strategy in Integrated Vector Management (IVM) and comes at a cost, depending on the strategy used. Use of commercial repellents has a direct cost that most poor people cannot usually afford in some countries, including those in Africa. Several studies documented different uses of mosquito repellents such as production of smoke by smoldering plants (Vernede et al., 1994), hanging up plants (Dube et al., 2011), application of plant oils and juices on the skin (Pavela & Benelli, 2016) and other methods used at local level. Some plants have insecticidal effect and eventually kill mosquitoes whereas others just ward off mosquitoes. Tabari et al., (2017) noted that some botanically based plants can provide eco-friendly vector control programs against mosquito vectors.



Ethnobotanical research documented use of plants and some researchers have gone further to validate claims associated with plant use. Validation of ethnobotanical plant extracts helps preserve effective plant species and discourage use of non-effective ones. Some validation processes have rendered useless some plants that were perceived to have mosquito repellent properties (Lalthazuali, 2017).

Moore and Lenglet (2004) proposed that the smoke from plants may disguise human kairomone cues used by mosquitoes to target their hosts or disrupt convectional currents responsible for location, and/or burnt leaves may release volatile compounds that repel or irritate mosquitoes. A study conducted by Paru *et al.*, (1995) in Papua New Guinea showed that wood smoke reduced Anopheline and Culicine biting by 79%. Deletre *et al.*, (2013) tested the efficacy of 20 plants on *Anopheles gambiae* adult mosquitoes and the following plants did not exhibit pronounced mortality ( $\geq$ 50%) when applied at 1% concentration; eucalyptus, pennyroyal, pepper, dill, ginger, neem, geranium, lemon, soldage, lemongrass, itsea, aframomum, colous, coriander and cumin. However, the following plants caused pronounced mortality on *An. gambiae* adult mosquitoes; citronella (88.6%), savory (87.3%) and thyme (87.9%).

The KT<sub>50</sub> (Knock down Time for 50% of *An. gambiae*) was recorded when plants were used at 1% concentration and the results were 1.2 (*Cymbopogon citrus*, 33.5 (*Cymbopogon giganteus*), 49.9 (*Cymbopogon schoenanthus*), 32.2 (*Eucalyptus citriodora*), 25.2 (*Eucalyptus tereticornis*), 8.1 (*Cochlospermuum tinctorium*), 20.9 (*Cochlospermuum planchonii*), 76.2 (*Securidaca longepedunnculata*) and 110.9 (*Chenopodium ambrosiodes*) (Bossou *et al.*, 2013). The KT<sub>90</sub> (Knock down Time for 90% of *An. gambiae*) when plants were used at 1% concentration was 6.6 (*Cy. citrus*), 100.2 (*Cy. giganteus*), 117.8 (*Cy. schoenanthus*), 61.7 (*E. citriodora*, 16.1 (*E. tereticornis*), 16.7 (*C. tinctorium*, 39.9 (*C. planchonii*), 91.3 (*S. longepedunnculata*) and 178.9 (*C. ambrosiodes*) (Bossou *et al.*, 2013).

The mortality rate when *An. gambiae* were used was 100% (*Cy. citrus*), 29.6% (*Cy. giganteus*), 6.7% (*Cy. schoenanthus*) 75.5% (*E. citriodora*), 98% (*E. tereticornis*), 72.7% (*C. tinctorium*), 24.5% (*C. planchonii*), 8.6% (*S. longepedunnculata*) and 41.9% (*C. ambrosiodes*) (Bossou *et al.*, 2013). All adult *Aedes aegypti* mosquitoes died when exposed for 120 minutes to *Lippia origanoides* that was used at 300 ppm (parts per million) (Castillo *et al.*, 2017). The study by Soonwera *et al.*, (2015) showed that the essential oil of *Cananga odorata* flowers knocked down 96% of *Ae. aegypti* mosquitoes as compared with 98.4% (*Anopheles dirus*) and 100% (*Culex quinquefasciatus*) mosquitoes. Plants have been shown to have repellent, knock down and insecticidal effect. Repellency alone is beneficial, but the ability to kill mosquitoes is a very big advantage.

The knock down and insecticidal effects of the plants *Tagetes minuta* (Mexican marigold), *Lippia javanica* (fever tea), *Lantana camara* (cherry pie), *Tagetes erecta* (African marigold) and *Eucalyptus grandis* (gum tree) were tested against *Anopheles arabiensis* mosquitoes in Mumurwi village, Bindura district, Zimbabwe.

# **Materials and Methods**

The leaves of the plants *T. minuta, L. javanica, L. camara, E. grandis* and *T. erecta* were collected from Mumurwi village (17°31'S - 22°31' E), Bindura district, Mashonaland Central province, Zimbabwe.

#### **Plant reparations**

After the leaves were collected, they were kept for 1 week at room temperature (26°C) for them to dry before grinding them to powder. A total of 1 kilogramme of eaves of each plant species was ground to powder using a pestle and mortar. Each plant species was kept separately in 1L jars away from sunlight until use.

#### **Preparation of huts**

A total of 6 grass thatched round huts used as kitchens were identified from Mumurwi village. Each hut had not been sprayed before using residual insecticides for malaria control. A smouldering fire using the same type of wood was smouldered in each of the 6 huts at the centre of the hut as traditionally done at the fire place. The round huts had wall heights measuring 1.98-2.1 metres with house diameters ranging 3.25-4.20 metres.

#### Knock down effect

A total of 20 female *An. arabiensis* mosquitoes were placed in each paper cup measuring 10.8cm (height), 7cm (top rim diameter) and 4.7cm (bottom rim diameter) and there were 5 paper cups for each treatment. The capacity of the paper cup was 250ml. The mosquitoes were acclimatized for 1 hour before use.

A total of 50 grams of powdered leaves of each plant species was placed in a metal tin that was placed on a smouldering fire in each of the selected thatched round huts. The control hut had the same conditions apart from the repellent plant. The mosquitoes that were knocked down in each paper cup were counted every 10 minutes for 140 minutes. The time required to knock down 50% ( $KT_{50}$ ) and 90% ( $KT_{90}$ ) of the mosquitoes was determined using the Environmental Protection Agency (EPA) Probit Analysis programme used for calculating LC/EC (lethal concentration/effective concentration) values, Version 1.5. This method was previously described by Finney, (1952).

### **Insecticidal effect**

All the mosquitoes that were used for the determination of knock down effect were provided with cotton swabs dipped in 10% sugar solution and eventually kept for 24 hours before scoring mortality.

#### Data analysis

The data was entered in an EXCEL sheet and analysed using ANOVA (Analysis of variance) method. Significant differences were determined at 95% confidence limit.

#### Results

### Knock down effect

The smoke from *T. erecta*, *T. minuta* and *E. grandis* knocked down 100% of the *An. arabiensis* mosquitoes within the 140 minute exposure time (Table 1). The smoke of *L. javanica* knocked down 100% of the mosquitoes 120 minutes post exposure but this efficacy was then lost. *L. camara* failed to knock down 100% of the *An. arabiensis* mosquitoes within 140 minutes. The mean knock down rate of *An. arabiensis* mosquitoes by *T. erecta* was higher than that by *T. minuta* although the results were not significantly different at 95% confidence limit (P=0.76). *T. minuta* knocked down more mosquitoes than *L. javanica* and the results were significantly different (P=0.93). *E. grandis* knocked down more mosquitoes than *T. minuta* although the results were not significantly different (P=0.99). *E. grandis* knocked down more mosquitoes than *L. camara* and the results were significantly different (P=0.028). *T. minuta* knocked down more mosquitoes than *L. camara* and the results were significantly different (P=0.027). The knock down rate of mosquitoes by *L. javanica* and *L. camara* were of the same magnitude and the results were not significantly different (P=0.93). All plants knocked down more mosquitoes than the control and the results were significantly different (P=1.35 ×10<sup>-10</sup>).

The plant *T. minuta* had the fastest  $KT_{50}$  followed by *E. gran*dis, *T. erecta* and *L. javanica* (Figure 1). The fastest  $KT_{90}$  was in this order: *T. minuta, E. grandis, T. erecta, L. camara* and *L. javanica.* Plants with fast knock down rate offer better protection from active mosquito biting than those with long knock down rates.

## Insecticidal effect of smoke

One hundred percent mortality of *An. arabiensis* mosquitoes was recorded with smoke from *T. erecta* and *E. grandis* (Table 2).



*T. minuta* killed <50% of *An. arabiensis* mosquitoes as compared with *L. javanica* and *L. camara*.

# **Discussions and Conclusions**

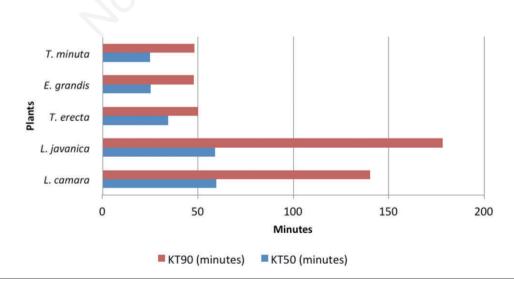
#### Knock down effect

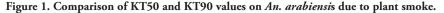
Smoke generated by repellent plants has been used to repel mosquitoes and insects. The knock down effect is one of the elements important in mosquito repellence since it shows, not only the ability to minimize mosquito human contact, but the potential to completely deactivate the biting possibility. In this study, the knock down effect of plants on mosquitoes increased with increasing time of exposure where *T. erecta*, *T. minuta* and *E. grandis* showed fast knock down on *An. arabiensis* mosquitoes. The ability

Table 1. The effect of smoke from plants in knocking down *An. arabiensis* mosquitoes (absolute numbers with % knock down indicated in brackets).

Minutes for exposure	<i>T. erecta</i> (%)	<b>T. minuta</b> (%)	L. javanica (%)	L. camara (%)	E. grandis (%)	Control (%)
10	10 (10)	0(0)	0 (0)	0 (0)	0 (0)	0(0)
20	40 (40)	32 (32)	10 (10)	0 (0)	30 (30)	0 (0)
30	69 (69)	70 (70)	30 (30)	0 (0)	70 (70)	0 (0)
40	100 (100)	76 (76)	10 (10)	30 (30)	78 (78)	0 (0)
50	100 (100)	93 (93)	50 (50)	10 (10)	92 (92)	0 (0)
60	100 (100)	100 (100)	50 (50)	50 (50)	100 (100)	1 (10)
70	100 (100)	100 (100)	80 (80)	79 (79)	100 (100)	1 (10)
80	100 (100)	100 (100)	41 (41)	79 (79)	100 (100)	1 (10)
90	100 (100)	100 (100)	70 (70)	79 (79)	100 (100)	1 (10)
100	100 (100)	100 (100)	79 (79)	82 (82)	100 (100)	1 (10)
110	100 (100)	100 (100)	81 (81)	88 (88)	100 (100)	1 (10)
120	100 (100)	100 (100)	100 (100)	89 (89)	100 (100)	1 (10)
130	100 (100)	100 (100)	88 (88)	73 (73)	100 (100)	1 (10)
140	100 (100)	100 (100)	75 (75)	89 (89)	100 (100)	1 (10)
Mean	87.1±28.2 <sup>a</sup>	$83.6 \pm 30.9^{a}$	$54.6 \pm 32.3^{b}$	$53.4 \pm 37.1^{b}$	83.6±31.1 <sup>a</sup>	$6.46 \pm 4.9^{\circ}$

The same letter in the same row means denotes no significant difference, different letter in the same row denotes significance difference.







of a plant based repellent to knock down mosquitoes fast is advantageous in that the number of mosquitoes taking a full blood meal and inflicting painful bites is minimized, thus having an effect in disease transmission.

It is worth noting that *T. erecta* had the fastest knock down effect (less minutes) whereby *L. javanica* took 3 times more than *T. erecta*. On the other hand, *E. grandis* and *T. minuta* took half the time to knock down 100% of the mosquitoes than *L. javanica*. Our results agree with other experimental researches, where smoke from burning vegetation or wood in Papua Nuova Guinea (Vernede *et al.*, 1994) and smoke from burning plants in South Africa (Mavundza *et al.*, 2011) were used for protection against mosquito biting. The study in Papua New Guinea showed that wood smoke reduced Anopheline and Culicine biting by 79% and this might be the case in our study were plants with fast knock down effect were found (Paru *et al.*, 1995).

Absence of complete knock down of *An. arabiensis* mosquitoes due to smoke from *L. camara* might be related to the exposure time. Exposure times more than 140 minutes might have either improved results or it might be that the plant *L. camara* reached its saturation peak and no further knock down could be recorded. Some element of reversal of mosquito knock down was experienced with *L. javanica* 130 minutes after exposure. Reversal of knock down has implications since this can increase the number of mosquitoes biting humans. Our results on 100% knock down of *T. erecta*, *T. minuta* and *L. javanica* compared well with those on *C. odorata* (*Cx. quinquefasciatus*) but better than *C. odorata* on *Ae. aegypti* and *An. dirus* by Soonwera *et al.*, (2015)

All plants in this study did not show very good  $K_{T50}$  like those observed with *Cy. citrus, C. tinctorium* and *C. planchonii* (Bossou *et al.*, 2013). However, the  $K_{T50}$  values of *T. erecta* were comparable to *Cy. giganteus* and *E. citriodora* (Bossou *et al.*, 2013). The  $K_{T50}$  values of *T. minuta* and *E. grandis* were comparable to *E. tereticornis* (Bossou *et al.*, 2013). The  $K_{T50}$  values of *L. javanica* and *L. camara* were higher than those of *Cy. giganteus* (Bossou *et al.*, 2013). Results on the  $K_{T50}$  values of all our plants were better than those of *S. longepedunnculata* and *C. ambrosiodes* (Bossou *et al.*, 2013).

In terms of  $K_{T90}$  values, *E. grandis* offered the fastest knock down rate, followed by *T. erecta*, then *L. camara* and lastly *L. javanica*. Plants with high knock down rates may be useful in minimizing human mosquito contact and therefore reduce mosquito nuisance. Such a scenario may improve the availability of peaceful nights by inhabitants with limited mosquito biting. These results indicate that plants may be used at a small scale in areas where these repellents are locally available.

## **Insecticidal effect**

The insecticidal effect of smoke generated by the plants *T. erecta* and *E. grandis* were well pronounced, killing 100% of the

Table 2. Number of dead mosquitoes 24hrs after exposure to plant smoke.

Plant repellent	24hr mortality of <i>An. arabiensis</i> (%)
T. minuta	40/100 (40)
T. erecta	100/100 (100)
L. javanica	75/100 (75)
L. camara	90/100 (90)
E. grandis	100/100 (100)
Control	0/100 (0)

An. arabiensis mosquitoes 24hrs after exposure and these results were better than those on citronella, savory and thyme (Paru et al., 1995). Our results on 100% mortality were also better than those on Cy. giganteus, Cy. schoenanthus, E. citriodora, E. tereticornis, C. tinctorium, C. planchonii, S. longepedunnculata and C. ambrosiodes (Bossou et al., 2013). Our results on 100% mortality of An. arabiensis exposed to T. erecta and E. grandis agree with findings by Castillo et al., 2017 on. L. origanoides .Such an observation may indicate a reduction in vector density to a limited scale and therefore making available very few mosquitoes for disease transmission. The fast knock down rates caused by T. erecta and E. grandis will be an added advantage when coupled with the high mortality rates as well. However, smoke from the plant T. minuta failed to kill a substantial number of An. arabiensis mosquitoes despite the high knock down rate experienced and these results are slightly higher than those recorded by Paru et al., (1995) on An. gambiae mosquitoes. The high knock down rate of the plant may be an advantage, since when knocked down mosquitoes fall on the floor; they can be picked up by scavengers and are eventually eliminated. The plants L. javanica and L. camara exhibited moderate insecticidal effect that may be useful in the prevention of insect biting, in agreement with findings by Liverani et al., (2017). Smoke generated by repellent plants might be useful in situations where commercial mosquito coils may be out of reach, taking into account that insect repellents are used at a large scale in countries like Mexico (Kuri-Morales et al., 2017). Our results may open up avenues for further research in the provision of alternative sources of mosquito repellents (Islam et al., 2017).

Plants used as mosquito repellents have shown time dependent activity on the knock down effect when their resultant smoke was assayed in village huts. The maximum exposure period of 140 minutes appeared to be ideal for 60% of the plants and there might be need to increase this time in order to take into account plants with slow knock down activity. In conclusion, smoke from the plants *T. erecta*, *T. minuta* and *E. grandis* had very fast knock down rates with *T. erecta*, *L. camara* and *E. grandis* killing over 90% of the *An. arabiensis* mosquitoes. Validation of the knock down and insecticidal effect of the plants *T. erecta*, *T. minuta*, *E. grandis*, *L. camara* and *L. javanica* has shown some promising results on their effect on *An. arabiensis* as noted by Lalthazuali *et al.*, (2017).

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