Original Article

Bulletin of Environment, Pharmacology and Life Sciences

Online ISSN 2277 – 1808
Bull. Environ. Pharmacol. Life Sci.; Volume 1 [10] September 2012: 16 - 20

Website: www.bepls.com

Laboratory Evaluation of Herbal Mosquito Coils against *Aedes aegypti* Mosquito

Deepak Phal[1, 2] Rajan Naik [1], Kedar Deobhankar[1], Swati Vitonde[1] and Nitin Ghatpande[1].
1. Ross Lifescience Pvt. Ltd., Pune, India.
2. Research Student of Department of Environmental Science, Singhania University, Rajasthan, India.
* Corresponding Author, E-mail: deepak@rosslifescience.com

ABSTRACT
Globally, different products in the form of biological, electrical and chemical sources are being used to control the menace of mosquitoes. In this study the attempts were made on coil product preparation using saw dust, rice husk, and corn cob based fillers along with herbal oils and herbal powders. In the present study, 17 herbal oils and 2 herbal powders were tested each at 15% concentration on the mosquito coil against Aedes aegypti in the Peet Grady chamber for its bioefficacy. Among these, only two oils viz pogastemon cabin and Acorus calamus exhibit significant knock down activity against Aedes aegypti. Further, these oils were screened at lower concentration i.e. 7.5% and 10 % concentrations respectively. The KT_{50} and KT_{90} values of each oil were determined. Attempts were also made to combine both these oils at different proportions and find out its efficacy. At 7.5% concentration Pogastemon oil and Acorus calamus (75:25) exhibit significant knock down activity. The implications of these observations are discussed.
It is also observed that, if the filler material is changed from sawdust to corncob or rice husk the bioefficacy results remain the same indicating that the filler material does not play any major role in efficacy.
KEY WORDS: *Mosquito, coil, knockdown, corn cob, rice husk, herbal oil and powder.*

INTRODUCTION

In developed countries the urban population is expected to increase to more than 70% [1] resulting in the increasing importance of urban pests. Similarly, for developing countries, the pests still remains as mosquitoes followed by others. Mosquitoes are the most important and abundant pest in urban, sub-urban and rural environment [2]. With the increasing problems caused by urban pests, there is an important need for their control. Insecticides have been heavily used as one of the most effective tools in vector control programmes. Although, chemical control provides quick knockdown/mortality, resistance of mosquito against the use of insecticides have been widely reported [3,4,5,6,7,8,9]. As such, more new insecticides/microbial agent have been developed in tropical countries like India. People use insect repellents products such as mosquito repellent coils, liquid vaporizers, mats, creams and aerosols sprays, for minimizing man mosquito contact and thereby preventing the menace of diseases like Malaria, Filariasis and Dengue caused by mosquitoes. These household insecticide products have been developed and are being used successfully in many parts of the world to prevent indoor mosquito biting [9, 10]. The Mosquito Coil when burnt continuously emits smoke along with the active ingredient added to it which prevents the mosquitoes from biting. This mode of release of insecticide for prevention of mosquito bites is an effective mosquito repellent tool [11]. Earlier, use of natural insecticide such as pyrethrum extract had been common in mosquito coil formulations but recently synthetic pyrethroids are gaining importance due to their efficacy against specific target insects and lesser toxicity to non targets. The use of mosquito coils have gained popularity in communities with both high and low malaria transmitting intensities as a supplement for protection from indoor mosquitoes, but outside it's still bed net [11]. India is a large consumer of Mosquito coils as compared to the electric products like mats and liquid vaporizers.

The present study was carried out to evaluate the herbal oils and powders as active material on coils prepared by using fillers such as sawdust, coconut shell powder, binders, sodium benzoate and potassium nitrate for its bioefficacy against *Aedes aegypti* under laboratory condition.

MATERIALS AND METHODS

Coils were prepared as per the standard procedure and sprayed uniformly with the herbal oils in different concentration (15, 10 and 7.5%) and these impregnated coils were used for its bioefficacy against *Aedes aegypti* mosquito in Peet Grady chamber. Initially, all these oils were evaluated at 15% concentration for its bioefficacy. The individual oils which were found to be active in terms of knock down effect were further selected for combination and with lower percentage of oil spray. Finally, coils manufactured from rice husk and corncob was also evaluated using with the selected oil combination.

Mosquito Coil

Mosquito coil was prepared using sawdust + coconut shell powder + jiggit + binder + sodium benzoate + potassium nitrate and individual herbal oil was sprayed (w/w) on top of the coil in different concentration of 15%, 10% and 7.5 % separately.

Test Chemical

Table No. 1: List of herbal oils and powder

Sr. No.	Scientific Name	Common Name	Chemical Ingredient
1	*Pogostemon cablin*	Patchouli	patchouli alcohol
2	*Acorus calamus*	Calamus / Sweet Flag	Alpha-asarone, Beta-asarone and eugenol
3	*Eucalyptus globulus*	Eucalyptus	p-Menthane-3,8-diol (95%)
4	*Ocimum basilicum*	*Basil*	Eugenol and Bornyl acetate
5	*Vitex negundo*	*Nirgundi*	α - Copaene and Camphene
6	*Curcuma longa*	*Turmeric*	ar-turmerone
7	*Calendula officinalis*	Calendula / Pot Marigold	α-cadinol
8	*Cinnamomum camphora*	*Camphor*	Camphor
9	*Mentha piperita*	*Pippermint*	Menthone
10	*Zanthoxylum limonella* fruits - Powder	Bajna / Bajrang - Powder	amides of *2E,6Z,8E,10E* dodecatetraenoic acid and *2E,4E,8Z,10E,12Z* tetradecapentaenoic acid
11	*Andrographis paniculata* - powder	Kalmegh - powder	andrograhpolide - a bicyclic diterpenoid lactone and Kalmeghin
12	*Pongamia glabra*	Karanja	High content of triglycerides, Karanjin
13	*Ricinus communis*	Castor	Ricinoleic acid
14	*Syzygium aromaticum*	Clove	Eugenol
15	*Trachyspermum copticum*	Ajwoin	Thymo
16	p-mentha-1,8-diene	R-limonene	(R)-1-methyl-4-(1-methylethenyl)
17	*Cymbopogon winterianus*	Citronella	3,7-Dimethyl-6-octenal, Geraniol, citronellal
18	*Thymus vulgaris*	Thyme	Thymol
19	*Zingiber officinale*	Ginger / Sunth	Gingerols

Herbal oils selected for the present study were obtained from different sources and local market. (as per the Table No. 1).

The coil was dried in the oven at 70^{0}c for 6 hours, and further kept in the room for half an hour of drying. Each oil with suitable diluents were sprayed (w/w) on the coil by using a hand spray pump. In case of herbal powder, it was incorporated in the coil ingredient.

The herbal oil premix was prepared by mixing 95% of herbal oil + 5% of deodorized kerosene and the premix was sprayed on the dry coil at 15% w/w concentration level. Finally these coils were packed

in a suitable container and kept for 2 – 3 days for storage. This is essential for the premix to spread uniformly on the coil.

Insect culture

Laboratory evaluation of these coils was done in Ross Lifescience Laboratory, which had the rearing and testing facility. The rearing of *Aedes aegypti* mosquito was under controlled temperature condition of $28^0 \pm 30^0$C and relative humidity of 60 – 80 %.

Test Arena

Peet Grady chambers of 5.8 m^3 made up of glass from 6 sides with controlled temperature and humidity conditions as per the protocol requirement (Busvine reference).

Bioefficacy test

The Peet Grady chamber was maintained with the temperature range of $28^0 \pm 30^0$C and humidity range of 60 - 80% for the entire experiment. 2-3 day old female *Aedes aegypti* mosquitoes were collected from the rearing cage and were released in the handling bottles. Before testing each product, the mosquitoes from the handling bottles were released into the Peet grady chamber and were kept in it for about 30 minutes. If the mortality exceeds 5% in the chamber then the chamber was to be cleaned and the above procedure was to be repeated. If mortality is below 5% the test is to be continued by replenishing only the dead insects. The coil was lit outside the chamber and was pushed inside the chamber up to the centre. The coil was allowed to smoulder continuously for 120 minutes observation period. The knockdown count was taken after every 5 minutes till 120 minutes using a hand-counter. The knockdown insects were collected and kept for 24 hours mortality observation in a jar with sucrose solution in it. Similarly, a test was done for the coil without herbal oils, (WHO reference)

The KT_{50} and KT_{90} values and percentage mortality after 24 hours of each coil was assessed using a minimum of two replicates

Similar experiment was conducted for control (without coil) to find the mortality of mosquitoes in the chamber. If the mortality exceeds 20% in the control batch, the results of the entire test was to be discarded. If the mortality in the controls was between 5 - 20%, the results with the coil samples were corrected using Abbott's formula [12].

$$\text{Mortality (\%)} = \frac{X-Y}{100-Y}\ X\ 100$$

Where X = percentage mortality in the treated sample and Y = percentage mortality in the control.

RESULT AND DISCUSSION

Results of the herbal oil premix at 15% concentration level against *Aedes aegypti* are documented in Table 2. It is clearly seen that, oils viz. Karanja, Castor, Clove, Ajwoin, limonene, Citronella, Thyme and Ginger didn't exhibit any knock down activity up to 120 minutes. However, Basil, Nirgundi, Turmeric, Pippermint oil, Bajna fruit powder and Kalmegh powder exhibit marginal activity, while 50% knock down was observed for more than 120 minutes. In case of Calendula oil and Camphor oil 50% (KT_{50}) knock down was observed within 100 mints and KT_{90} values were more than 120 mints. Among these oils, Patchouli oil exhibit significant activity followed by Calamus and Eucalyptus.

On the basis of results obtained (Table 2), active oils are further evaluated at lower concentration i.e. 10 and 7.5% for its bioefficacy and presented in Table 3

It was observed that the knock down activity of mosquito at 7.5% concentration level is slightly higher time as compared to 10% and 15% concentration. However, combination of herbal oils (Patchouli : Calamus) at two different percentages viz (50:50) and (75:25) were selected for further evaluation. It was observed that decrease in Patchouli oil from 100% to 50% in the combination mixture the KT value increases. In case of 75% of Patchouli oil and 25% of Calamus oil the KT value is more or less similar to 10% concentration.

Spraying the coils with herbal oil at 15% concentration makes them damp / soggy. Due to this problem, 7.5% and 10% concentrations were selected for further study. At 7.5% concentration higher knock down activity (KT values) was observed as compared to 10% concentration. However, a combination product [Patchouli + Calamus (75:25)] at 7.5% exhibits similar efficacy with compare to 10% concentration. (Table 3).

Table No. 2. : Bioefficacy of different herbal oils and powders (and their combination) at 15% concentration.

Herbal oil / powder	% used in coil	Percentage knockdown time (minutes)	
		KT_{50}	KT_{90}
Patchouli	15% Oil Spray	37.06	67.20
Calamus		44.86	76.49
Eucalyptus		52.91	88.99
Basil		>120	>120
Nirgundi		>120	>120
Turmeric		>120	>120
Calendula		96.35	>120
Camphor		72.11	>120
Pippermint		>120	>120
Karanja		No Activity	
Castor		No Activity	
Clove		No Activity	
Ajwoin		No Activity	
d-limonene / orange peel		No Activity	
Citronella		No Activity	
Thyme		No Activity	
Ginger		No Activity	
Bajna - Powder	15% powder in coil	>120	>120
Kalmegh - powder		>120	>120
Blank coil (without herbal active)	--	0	0

Table No. 3: Bioefficacy results of coils with selected herbal oils at 10% and 7.5% concentration level

Herbal oil / powder	Premix oil spray % used on coils	Percentage knockdown time in minutes	
		KT_{50}	KT_{90}
Patchouli	10%	44.72	74.62
Calamus		56.57	88.83
Patchouli	7.50%	52.91	87.69
Calamus		65.35	106.77
Patchouli + Calamus (50:50)		76.03	123.51
Patchouli + Calamus (75:25)		44.81	78.46
Patchouli + Calamus + Eucalyptus (75:12.5:12.5)		48.52	83.73

The corn cob / rice husk based coil prepared by replacing the saw dust completely and sprayed with 7.5% combination of Patchouli + Calamus (75:25). The results showed similar efficacy for all sawdust coil, rice husk coil and corn cob coils. This means if the coil filler material is changed there is no impact on overall efficacy of the product. (Table 4)

Table No. 4 : Bioefficacy results of coils with selected herbal oils at 7.5% concentration level and different filler material

Coils prepared using filler as	Herbal oil / powder	Percentage knockdown time in minutes	
		KT_{50}	KT_{90}
Saw dust powder	Patchouli + Calamus (75:25)	44.81	78.46
Corn cob powder		44.99	77.55
Rice husk powder		45.84	79.01

CONCLUSION

It is evident from the present study that, combination product Patchouli + Calamus (75:25) exhibit significant knock down activity at 7.5% concentration with all the coils (rise husk, corn cob and saw dust based coils). These coils were further tested and analyzed in terms of burn time, length, width, thickness, tensile strength and breakability and was found to be within range for all the filler material (saw dust, corn cob and rice husk). Further simulated and actual field trials required for commercialization of these herbal mosquito coils.

ACKNOWLEDGEMENTS

I would like to thank the Department of Environmental Science, Singhania University, Rajasthan for allowing me to carry out this research work, and my sincere thanks to Ross Life Science Pvt. Ltd. for allowing me to generate bioefficacy data from their lab. The authors are thankful to the Dr.S.G.Deshpande, National Chemical Laboratory, Pune for his guidance.

REFERENCES

1. Watson C. (1993). Trends in world urbanisation. In, Wildey K.B. and Robinson W.H.(eds.), *Proceedings of The 1st International Conference On Insect Pests In The Urban Environment. Cambridge University, UK*
2. Lee C.Y. and H.H. Yap. (2003). Status of urban pest control in Malaysia. In, Lee CY, Yap HH, Chong N.L, and Jaal Z. (eds.), *Urban Pest Control, A Malaysian Perspective. Universiti Sains Malaysia. 1-8 pp.*
3. Omer S.M., G.P. Georghiou and S.N. Irving. (1980). DDT/pyrethroid resistance inter-relatioships in *Anopheles stephensi*. *Mosquito News* 40:200-209.
4. Poovaneswari S. and S.K. Lam. (1992). Problems in dengue control: a case study. *Southeast Asian Journal of Tropical Medicine and Public Health* 23:723-726.
5. Raymond M., N. Pasteur and G.P. Georghiou. (1987). Inheritance of chlorpyrifos resistance in *Culex pipiens* L (Diptera: Culicidae) and estimation of the number of genes involved. *Heredity* 58:351-356.
6. WHO [World Health Organization]. (1964). Genetics of Vectors and insecticide resistance. *WHO/TRS/268. WHO, Geneva, Switzerland.40 pp.*
7. WHO [World Health Organization]. (1980). Resistance of vectors of disease to pesticides, fifth report of the WHO expert Committeeon Vector Biology and Control, Technical Report Series, No 655, *WHO, Geneva, Switzerland, 82 pp*.
8. WHO. (1992). Vector resistance to pesticides, fifteen report of the WHO expert Committee on Vector Biology and Control, Technical Report Series, No 818, WHO, *Geneva, Switzerland. 62 pp.*
9. Wirth M., G.P. Georghiou, N. Pasteur and L.L. Luna. (1987). Evolution of resistance and change in relative density in a *Culex tarsalis* (Diptera:Culicidae) population under heavy insecticidal control. *Journal of Medical Entomology* 24:494-497.
10. Birley, M. H., Mutero, C. M., Turner, I. F. and Chadwick, P.R. (1987). The effectiveness of mosquito coils containing esbiothrin under laboratory and field conditions. *Ann. Trop. Med. Parasitol., 81:163-171*.
11. C.H. Wang, (1993). Application of dosage technology on insecticides for household use. The *Bulletin of Environmental Health 1, 23-37 (in Chinese)*
12. Abbott WS. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18, 265–267.

QR CODE: T100178
http://www.bepls.com

BEPLS ABSTRACTED AND INDEXED

Zoological Records [USA, Thompson Reuters], ISI Master Journal List, Index Copernicus, EJournal, WorldCat, ABC Open Directory, Newjour, Geneva Medical Foundation, Electronic Journal Library, Global Education Index, Indiawaterportal, Valiasr, Google, Google Scholar and listed in many more libraries.