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Efficacy of *Adhatoda zeylanica* Medik. leaf as mosquito repellent

Shreya Medda, Uttiya Dey, Deep Chakraborty, Jayanta Kumar Datta and Naba Kumar Mondal*

Department of Environmental Science, The University of Burdwan
Burdwan-713104, West Bengal, India

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Herbal products are cheaper and effective than chemical based mosquito repellent, therefore an attempt has been made to prepare a 100 % herbal product based on traditional practices and rural wisdom. Present study was conducted for effective utilization of Basak (*Adhatoda zeylanica* Medik. syn. *A. vasica* Nees) as a mosquito repellent. Seven varieties of products were prepared from its leaves with different natural compositions. Study results revealed that maximum percentage of weight loss with V_7 variety followed by V_1 and lowest in variety V_6. Almost all varieties of bio-cake burned within 35 – 45 minutes except variety V_5, which took 60 minutes to complete burning. Variety V_6 showed highest ash content (7.70 g) and lowest ash was recorded for V_5 (1.67 g) after complete burning. The emission of CO_2, CO and O_3 was recorded as $V_2>V_4>V_3>V_5 \approx V_7>V_1>V_6$; $V_5>V_6>V_1>V_7>V_4>V_2>V_3$ and $V_5>V_3 \approx V_4 \approx V_7>V_2>V_1>V_6$, respectively during burning of bio-cake. On the other hand knock down experiment demonstrated that almost all varieties of bio-cakes are effective as mosquito repellent. But only two varieties (V_6 and V_7) caused more than 50 % knock down. Finally the emission of gaseous product was compared with a synthetic mosquito coil.

Keywords: Basak, *Adhatoda zeylanica,* Biocake, Mosquito repellent, Gaseous product, Synthetic coil.
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Introduction

Controlling mosquitoes is important in the present day scenario with rising number of mosquito borne diseases. An alarming increase in the range of mosquitoes is mainly due to deforestation, industrialized farming and stagnant water. Thus, special products like mosquito repellents for combating mosquitoes are required. The products used for mosquito control have varying degrees of effectiveness. Carbon dioxide and lactic acid present in sweat in warm-blooded animals act as an attractive substance for mosquitoes because perception of the odour is through chemo receptors present in the antennae of mosquitoes. A number of natural and chemical mosquito repellents, published in research papers[1-8], are mainly effective as to repel mosquitoes. People use insect repellents such as mosquito repellent synthetic 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. 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 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 its still bed net[11]. India is a large consumer of mosquito coils as compared to the electric products like mats and liquid vaporizer. Natural pyrethrins from insecticidal pyrethrum extract and synthetic pyrethroids such as allethrin, d-trans allethrin, prallethrin, transfluthrin, cyfluthrin, deltamethrin and permethrin are active ingredients in insecticidal formulations such as powders, sprays, impregnated papers, electro evaporators and mosquito coils intended for indoor use[12]. Residues of pyrethroids in air and their deposition on surfaces suggest the

*Correspondent author:
E-mail: nkmenvbu@gmail.com
Mob.-+91- 9434545694

possible exposure of humans due to inhalation or skin absorption [13]. All the experimental plant species have immense utility in the field of medical science, perhaps due to presence of important chemical constituents. The medicinal use of experimental plant species are: *Alpinia galangal* Willd. (bronchitis, ulcers, cholera, etc.); *Boesenbergia pandurata* Roxb. (antipyretic, anti-inflammatory, insecticidal etc.); *Curcuma longa* L. (digestive disorder, arthritis, cancer, etc.); *Elettaria cardamomum* Maton (anti-cancer, dermatological effects, antiulcer effects, etc.); *Azadirachta indica* A. Juss. (fungal infection, skin treatment, antioxidant, etc.); *Cassia siamea* Lam. (stomach complaints, purgative properties, etc.); *Cymbopogon nardus* (L.) Rendle (tropical insect repellent, head lice, anti-bacterial activity; *Eucalyptus citriodora* Hook. (anti-inflammatory, pulmonary effect, insect repellant) and *Eupatorium odoratum* L. (skin disease, memory loss, fever malaria). The nine potential plants namely greater galangal (*Alpinia galangal* Willd.), finger root (*Boesenbergia pandurata*), turmeric (*Curcuma longa*), cardamom (*Elettaria cardamomum*), neem (*Azadirachta indica*), siamese cassia (*Cassia siamea*), citronella grass (*Cymbopogon nardus*), eucalyptus (*Eucalyptus citriodora*) and siam weed (*Eupatorium odoratum*) that expressed high degree of repellency against mosquitoes are recommended as new active ingredients for inclusion in mosquito coil formulations[1].

To overcome the some toxic effects from the smoke of synthetic mosquito repellents, present study was carried out to evaluate the efficacy of *Adhatoda zeylanica* leaf as an active ingredient towards the mosquito repellent. Best performance (knock down) of prepared biocakes was compared with the synthetic mosquito coil with respect to generation of CO and CO_2 during burning. Basak leaves in the form of cakes are seems to be the first and preliminary report.

Materials and Methods

Collection of *A. zeylanica* leaves after steam distillation

Basak (*A. zeylanica*) leaves used for present study were collected and processed through steam distillation. The steam distillation is commonly used to extracts chemicals from plant materials. In this process pressure is reduced inside the flask and low temperature is needed for heating which protects organic compounds from its decomposition. After steam distillation leaves were grounded into paste using motor pestle by adding distilled water. 40 g of basak leaves paste was taken and was plated accordingly (Plate 1). Both wet weight and dry weight was taken after 24 h suns drying. Cut leaf pieces were grounded into paste using electrical grinder by adding distilled water. The different natural binders (50 g each) were purchased commercially from local vendors such as wood powder, potato starch, corn starch, coconut shell powder, neem powder and cow dung. Each cake was prepared as 20 % (w/w) binder (i.e. wood powder, coconut shell powder, neem powder, cow dung, cornstarch, potato starch) along with the paste of basak leaf (40 %) for all variety of cakes. As a result, a total of seven cake formulations comprising different binders with basak leaves as active ingredient were prepared for testing including

Plate 1—Different varieties of biocake

one synthetic coil such as V_1: *A. vasica* leaf paste; V_2: *A. vasica* leaf paste + 20 % wood powder; V_3: *A. vasica* leaf paste + 20 % neem powder ; V_4: *A. vasica* leaf paste + 20 % potato starch ; V_5: *A. vasica* leaf paste + 20 % corn starch; V_6: *A. vasica* leaf paste + 20 % cow dung; V_7: *A. vasica* leaf paste + 20 % coconut shell powder and V_8: synthetic coil (0.1 % (w/w) d-trans Allethrin + other ingredients 99.9 % (w/w). Basak leaves cakes with no supplementation were used as reference cake. Wet weight and dry weight of each cake were taken after 24 h of drying[1].

Evaluation of mosquito repellent activity

For investigating mosquito repellent activity the prepared cakes were checked for its flammability, burning efficiency with respect to burning time and eventually its effective repellent activity (Fig. 1). Flammability test of these cakes were conducted to check its consistent combustibility. Ash produced by cakes were weighed and recorded in Table 1. Further the time taken to burn the cake, smoke produced and production of CO, CO_2 and O_3 were observed and recorded (Table 2). The cakes were burned in selected mosquito prone areas in the evening and night period such as bushes, shrubs, laboratory corners, department premises and cafeteria.

Results and Discussion

From the Table 1 it is observed that, all the combination products of *A. zeylanica* leaf, significantly vary from one another except V_4 and V_5 with respect to fresh and dry weight. The variety V_7 showed highest wet weight and it is significantly different ($p< 0.05$) from other wet weight followed by variety V_3 and V_6 and lowest in variety V_5 (Table 1). However, highest dry weight was recorded for variety V_6 followed by V_4, V_7 and V_3 and lowest in Variety V_1. Therefore, it is easily calculated their percentage of weight loss and results suggested that there was maximum weight loss in variety V_7 and lowest in variety V_4. Therefore, so far as biomass is concerned variety V_7, V_1, V_2 and V_3 required large biomass for preparation of biocake compared to the other variety such as V_4, V_5 and V_6. On the other hand burning time data suggested that variety V_5 took maximum time (60 minute) to complete burn a biocake followed by variety V_6 (45 minutes) and lowest time taken by variety V_1 and V_7 (35 minutes). However, variety V_2, V_3 and V_4 does not show significant variation in burning time (Table 1). The production of ash is very important so far as waste product is concerned. Present study results revealed that minimum weight of ash was produced from variety V_5 (1.6 g) after complete burning of a dry cake (weight ranges from 9.54 to 21.15 g) followed by variety V_2 (2.12 g) and highest ash produced from variety V_6 (7.70 g). Such high level of ash content was reported by other investigator[14].

During burning of biocake ash may not be the only product, but other gaseous product is also considered

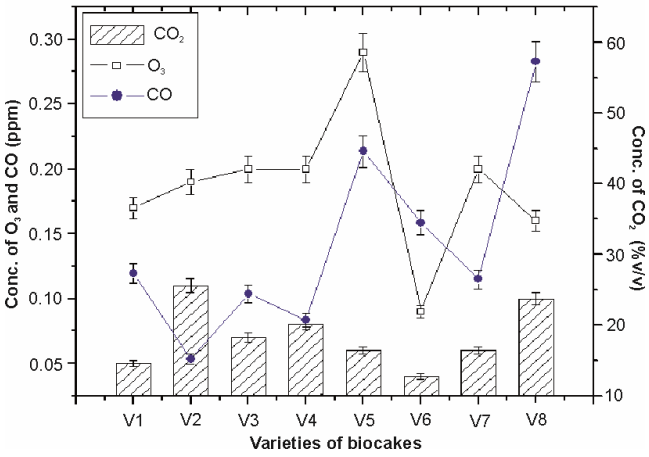

Fig. 1—The concentration of CO (ppm), CO_2 (ppm) and O_3 (ppm) during burning of biocake

Table 1—Wet and dry weight, % of wet loss, time taken for complete burning and ash weight of prepared biomass

Varieties of biocake	Wet weight(g)	Dry weight (g)	% of weight loss (g)	Burning time (min)	Ash weight (g)
Adhatoda zeylanica leaf paste (V_1)	50.16±0.335	9.54±0.457	80.98±0.572	35±1.45	2.8±0.371
Leaf paste+20% wood powder (V_2)	70.20±0.239	16.25±0.409	76.85±0.506	40±0.333	2.12±0.232
Leaf paste+20% neem powder (V_3)	66.99±0.857	18.35±0.229	72.60±1.25	40±0.333	3.60±0.378
Leaf paste+20% potato starch (V_4)	50.18±0.255	20.23±1.13	59.68±0.28	40±0.333	2.88±0.56
Leaf paste+20% corn starch (V_5)	50.15±0.509	15.95±0.92	68.19±0.544	60±0.333	1.67±0.561
Leaf paste+20% cow dung (V_6)	64.17±0.492	21.15±0.185	67.04±0.712	45±0.333	7.70±0.371
Leaf paste+20% coconut shell powder (V_7)	111.49±0.433	18.17±0.635	83.70±0.524	35±0.333	2.57±0.367

±SE Mean

Table 2—Correlation between wet weight, dry weight, % of weight loss, time, ash weight, CO_2, CO and O_3

	Wet weight	Dry weight	% of Weight loss	Burning time	Ash weight	CO	CO_2
Dry weight	0.255						
% of Weight loss	0.611	-0.581					
Burning time	-0.425	0.151	-0.494				
Ash weight	-0.024	0.484	-0.282	-0.05			
CO	0.03	0.53	-0.021	-0.133	-0.541		
CO_2	-0.219	-0.025	-0.189	0.773 *	0.174	-0.704	
O_3	-0.102	-0.233	-0.024	0.505	-0.848	0.301	0.268

such as carbon monoxide, carbon dioxide and ozone. Study results revealed that maximum CO was discharged during burning of variety V_5 (44.6 ppm) followed by variety V_6 (34.5 ppm) and lowest in variety V_2 (15.2 ppm). However, status of carbon dioxide showed opposite picture for variety V_2 (0.11 ppm) which produced maximum carbon dioxide followed by variety V_4 (0.08 ppm) and V_3 (0.07 ppm) and lowest CO_2 discharged from variety V_6 (0.04 ppm) (Fig. 2). Study results also suggest that cake burning smoke is also responsible to produce very small amount of ozone and this particular gaseous component is only considered during day time observation. Ozone concentration was highest in variety V_5 (0.29 ppm) followed V_3, V_4 and V_7 having same concentration of ozone (0.20 ppm) and lowest in variety V_6 (0.09 ppm). The correlation study suggest that dry weight is positively related with ash content (r = 0.484) and carbon monoxide (r = 0.53). However, negatively related with carbon dioxide (r = -0.025) and O_3 (r = -0.233) production (Table 2). On the other hand, time of burning showed significant positive relationship with carbon dioxide (r = 0.773, $p < 0.01$) and ozone (r =0.505). The ash weight and carbon monoxide showed significant negative relationship with ozone (r = - 0.848, $p<0.01$) and carbon dioxide (r = - 0.704 $p<0.01$), respectively (Table 2).

Finally the knock down test showed positive results for all the varieties of prepared biocakes but best result was recorded for variety V_6 (*A. zeylanica* leaf paste + 20 % cow dung) and V_7 (*A. zeylanica* leaf paste + 20 % coconut shell powder) which showed 70 % knock down after complete burning of a biocake. However, after exposed with the variety V_1, V_3 and V_2 showed unusual behaviors such as way from smoke, unable to fly, etc. among the tested mosquitoes. But none of the varieties showed excellent performance as synthetic one (V_8) (100 %).

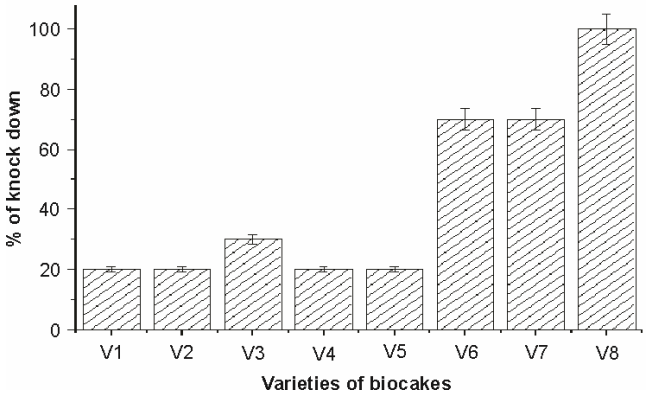

Fig. 2—Percentage of knock down tested with synthesized biocake and synthetic mosquito coil

Conclusion

The Present study revealed that biocake prepared from Basak leaves possesses potentiality towards repellent activity of mosquito as well as the importance of its leaf remains which are generally thrown as waste. In addition, the use of other natural products in the mixture, such as binders, could increase the protection time, potentiating the repellent effect of some essential oils. On the basis of results obtained, it could be concluded that the basak leaf cakes with corn starch (20 %) as a binder showed maximum burning time (60 min), minimum ash weight (1.67 g) and moderate CO_2 level. However, biocake V_6 and V_7 showed maximum knock down but less than V_8. This report is the preliminary work done using basak leaf cakes as herbal mosquito repellents alone with natural binders. However, more extensive research is required to explore the potentiality of other herbal product with respect to the mortality of mosquito.

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References

1 Rani N, Wany A, Vidyarthi A S and Pandey D M , Study of Citronella leaf based herbal mosquito repellents using natural binders, *Curr Res Microbiol Biotechnol*, 2013, **1** (3), 98-103.

2 Kim J K, Chang S K, Jong K L, Young R K, Hye Y H and Hwa K Y, Evaluation of repellency effect of two natural aroma mosquito repellent compounds, citronella and citronellal, *Entomol Res*, 2005, **35**(2), 117-120.

3 Mandavgane S A, Pattalwar V V and Kalambe A R, Development of cow dung based herbal mosquito repellent, *Nat Prod Rad*, 2005, **4** (4), 270-273.

4 Nerio L S, Olivero-Verbel J and Stashenko E, Repellent activity of essential oils: A review, *Biores Technol*, 2010, **101**, 372-378.

5 Patel E K, Gupta A and Oswal R J, A review on mosquito repellent methods, *Int J Pharm Bio-Sci*, 2012, **2**(3), 310-317.

6 Phal D, Patil S, Naik R, Deobhankar K, Vitonde S and Ghatpande N, Concentration of d-trans allethrin in air after complete smoldering of mosquito repellent coil manufactured using different fillers, *Int J Biol Pharm Allied Sci*, 2012, **1**(9), 1312-1321.

7 Tawatsin A, Thavara U and Chompoosri J, Field evaluation of mosquito coils derived from plants against night-biting mosquitoes in Thailand, *Proceedings of International Conference on Biopesticides,* 2002, **3**, 243-253.

8 Wratten S D, Scott R R, Thavara U and Techadamrongsin Y, Repellency of volatile oils from plants against three mosquito vectors, *J Vector Ecol*, 2001, **26**, 76-82.

9 Wirth M, Georghiou G P, Pasteur N and Luna L, Evolution of resistance and change in relative density in a *Culex tarsalis* (Diptera: Culicidae) population under heavy insecticidal control*, J Med Entomol*, 1987, **24**, 494-497.

10 Birley M H, Mutero C M, Turner I F and Chadwick P R, The effectiveness of mosquito coils containing esbiothrin under laboratory and field conditions, *Ann Trop Med Parasit*, 1987, **81**, 163-171.

11 Wang C H, Application of dosage technology on insecticides for household use, *Bull Environ Health*, 1993, **1**, 23-37.

12 Troost J R, An air to water bridge: air sampling and analysis using tetraglyme, *Anal Chem*, 1999, **71**, 1474-1478.

13 Ramesh A, Rapid pre-concentration method for the determination of pyrethroid insecticides in vegetable oils and butter fat and simultaneous determination by gas chromatography-electron capture detection and gas chromatography-mass spectrometry, *Analyst*, 1998, **123**, 1799-1802.

14 Phal D A, A comparative study of element analysis in mosquito repellent coil and cigarette smoke, *Int J Pharm Biol Sci*, 2011, **1**(4), 462-467.