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Novel method for screening Mosquitocidals against *Aedes aegypti*

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Abstract
Aedes aegypti is known to transmit viruses *viz*. Dengue, Yellow Fever, Chikungunya, Zika etc. Present paper describes novel method for quick shortlisting of the promising active moieties from ocean of the botanically derived extracts, essential oils, crude powder, etc. By conventional method, maximum of two to three experiments can be carried out daily in Peet Grady Chamber (5.83 M^3). On the contrary, with this novel method we can carry out 7-10 experiments daily thereby saving time and energy. Only selected promising efficacious ones from novel method will be evaluated by conventional method in Peet Grady Chamber (5.83 M^3). Thus, current method will save time, manpower and mosquitoes required by conventional method.

Keywords: Mosquitocidals, aedes, peet grady chamber

1. Introduction
Pyrethrum, Nicotine and Rotenone were identified as effective insect control agents in 17th century. Natural Pyrethrum, isolated from the flower heads of pyrethrum (*Chrysanthemum cineraria folium*), is one of the important insecticides widely used in mosquito control. However, Pyrethrins are unstable in the light and are rapidly metabolized which limit their potency and application [3] which led to development of synthetic analogues, termed as synthetic pyrethroids. Synthetic pyrethroids spread easily and rapidly because of its special attributes like quick action and easy availability with longer shelf life. Subsequently, it was noticed that these synthetic insecticides come with ever increasing costs, develop resistance among pests and possible toxicity to non-target organisms. After facing problems due to over application and injudicious use of synthetic insecticides in nature, refocus shifted on botanical pesticides that are easily biodegradable, with no residual effects and having no ill effects on non-target organism was appreciated [12]. The number of the plants having insecticidal substances is enormous i.e. more than 2000 plant species are known to possess insecticidal bioactivity [2]. The flowering plant families *viz*., Asteraceae, Fabaceae, Lamiaceae found to contain many insecticidal plant species.
In 2003, Indian council of medical research published a bulletin entitled Prospects of using herbal products in the control of mosquito vectors compiled all the plants reported from 1990 to 2002, covering range of bio-efficacies *viz*., insecticidal, growth inhibition and repellent activity against mosquito vectors [4].
Several studies have reported detailed information on phytochemicals with mosquitocidal potential and state of knowledge on larvicidal plant species, extraction processes, growth and reproduction inhibiting phytochemicals, herbal ovicides, synergistic, additive and antagonistic joint action effects of mixtures, residual capacity, effects on non-target organisms, resistance, screening methodologies [5, 10]. Plants belonging to family Meliaceae has been reported to have potential larvicidal activity, hence, feasibility of using those plant extracts for control of larva in field condition was studied in order to use cheap alternative to the conventional larvicides. [13]. Major components of each botanical sample may contribute to its larvicidal, repellency and adulticidal effects and while developing alternative insecticide, factors such as safety of non-target beneficial organisms, their resistance potential and their residual life span should be considered [6].

From well-known mosquito species, *Aedes aegypti* is known to transmit viruses *viz*. Dengue, Yellow Fever, Chikungunya, Zika etc. Present paper describes novel method for quick shortlisting of the promising active moieties from ocean of the botanically derived extracts, essential oils, crude powder, etc. Main objectives to devise current novel method were; 1. Efficacy of any moiety is conventionally established in Peet Grady Chamber (5.83M^3) [7, 8] that requires proper cleaning for ensuring absence of residual contamination, which is time consuming process. Thus, each experiment run requires at least 210 minutes comprising, Thirty minutes for Control experiment to confirm absence of residual contamination. Actual Test Experimental time is 60 minutes. Thirty minutes for removing previous experiment smoke/ vapors/ air and for collecting knocked down mosquitoes. Sixty minutes for washing with Iso-propyl alcohol and drying require 30 minutes. 2. By conventional method, maximum of two to three experiments can be carried out daily. On the contrary, with this novel method we can carry out 7-10 experiments daily thereby saving time and energy. 3. Only selected promising efficacious ones from novel method will be evaluated by conventional method in Peet Grady Chamber (5.83 M^3). Thus, current method will save time, manpower and mosquitoes required by conventional method.

2. Materials and Methods

2.1 Insects

Aedes aegypti used in this bioassay were cultured in permanent colony of Ross Lifescience Limited, Pune, Maharashtra, India. The mosquitoes were fed with 10% sugar solution from the day of emergence to the day of testing and reared at 27 °C and 60 ± 10% relative humidity.

2.2 Standards

Smouldered 0.3g of 0.1% d-trans allethrin Market coil (Standard) in a system A, thereby releasing 0.3mg of d-trans allethrin. Batch number: 17025. Manufactured at Bharat Health Care Products, Hyderabad, Andhra Pradesh, India.

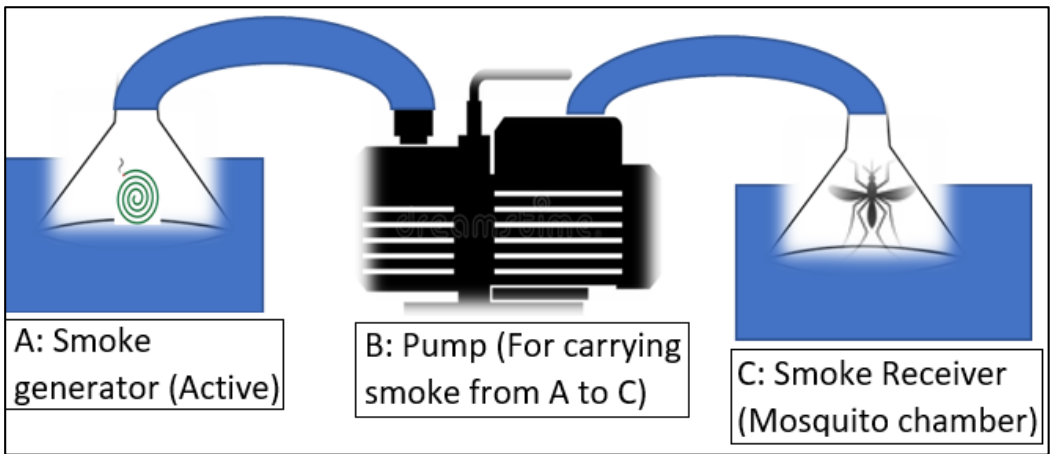
2.3 Test Samples

Test samples were essential oils which were supplied by following Indian Essential Oil suppliers with Batch number and its Certificate of Analysis. The essential oils sourced from Uttar Pradesh based firms like; AG Industries, Aromaaz International, VDH Organics, Venkataramana industries, Ultra International; Maharashtra based firms like; S-7 Solutions, Pune, JK Herbs, Pure Herbs and Anant Herbs, Mumbai, Maharashtra and Karnataka based firms like; Falcon & Novosynth. Test samples were selected basis results of following publications available in literature [9, 4, 6, 2, 11, 5].

2.4 Method Set Up

1. Control Experiment: Smoldered blank coil in a System A. Generated smoke is taken to System C with the help of pump (System B) which is connected to both the System A for aspiration of smoke and to System C for releasing the Smoke.
2. System A is for generating the active for 10 minutes (in the form of smoke)
3. System C is for containing free flying mosquitoes and observing the knock down as an impact of smoke for 30 minutes.
4. System B is installed for conveying the smoke from System A to System C for 10 minutes.
5. Test sample Experiment: Blank coil tip is impregnated with 30mg neat essential oil and smoldered. Repeated above process serial number 1 for test substance evaluation.
6. Standard Experiment: Market coil with 0.1% Esbiothrin coil was smoldered for 10 minutes and checked knock down for 30 minutes.
7. Determined KT_{50} and KT_{95} by Probit Analysis method (Finney, 1971) which is commonly used in toxicology to determine the relative toxicity of chemicals to living organisms. This is done by testing the response of an organism under various concentrations of each of the chemicals in question and then comparing the concentrations at which one encounters a response.

2.5 Model



Raw knock down data (*Aedes* mosquito response to smoke for 10 minutes and impact of residual efficacy for 20 minutes) was collected at every minute interval up to 5 minutes then subsequently 5, 10, 15, 20, 25 up to 30 minutes. Thus, generated huge data subjected to probit analysis to determine KT_{50} and KT_{95} to understand the efficacy aspect of the evaluated moieties. Thus, KT_{50} and the KT_{95} are nothing but the time required for knocking down 50% and 95% population respectively. The data depicted below in terms of KT_{50} and KT_{95} values are the outcome of three replicates against each moiety.

Table 1: KT_{50} and KT_{95} values for given sample name.

Sr. No.	Items	KT_{50} (min.)	KT_{95} (min.)
1	Market Anti-Mosquito coil (0.1% Esbiothrin)_ Standard	1.9	3.5
2	*Tanacetum annuum*	1.9	3.5
3	*Pogostemon cablin*	2.0	3.9
4	*Copaifera langsdorffii*	2.1	3.7
5	*Cuminum cyminum*	2.1	3.7
6	*Cedrus deodara*	2.4	4.0
7	*Achillea millefolium.*	2.7	5.1
8	*Artemisia absinthium*	2.7	5.1
9	*Helianthus annus*	2.7	5.1
10	*Eucalytpus globulus*	3.0	5.5
11	*Ocimum gratissimum*	3.0	5.2
12	*Trichospermum ammi*	3.0	5.2
13	*Ocimum sanctum*	3.1	5.5
14	*Origanum vulgare*	3.1	5.3
15	*Chamaemelum nobile*	3.1	5.4
16	*Ocimum basilicum*	3.2	5.8
17	*Myrocarpus fastigiatus*	3.3	5.7
18	*Trachyspermum copticum*	3.3	5.7
19	*Lavandula spp*	3.3	5.6
20	*Pistacia terebinthus*	3.3	5.6
21	*Anthemis nobilis*	3.5	6.1
22	*Mentha citrate*	3.5	6.1
23	*Anethum graveolens*	3.6	5.8
24	*Artimisia dracunculus*	3.6	5.8
25	*Curcuma zedoria*	3.6	5.8
26	*Hyssopus officinalis*	3.6	5.8
27	*Vitex agnus-castus*	3.6	5.8
28	*Mentha spicata*	3.6	5.9
29	*Vetiveria zizanoids*	3.6	5.8
30	*Artemisia vulgaris*	3.8	6.9
31	*Cretan oregano*	3.9	6.9
32	*Salvia sclarea*	3.9	6.5
33	*Tagetes erecta*	3.9	6.5
34	*Cinnamomum tamla*	3.9	6.2
35	*Citrus lemon*	3.9	6.2
36	*Abelmoschus moschatus*	4.1	6.5
37	*Myroxylon pereirae*	4.1	6.5
38	*Mentha piperita*	4.1	6.9
39	*Inula graveolens*	4.1	7.0
40	*Melissa officinalis*	4.1	7.1
41	*Lavandula officinalis*	4.4	7.0
42	*Citrus limon*	4.4	7.3
43	*Rosmarinus officinalis*	4.4	7.3
44	*Trigonella foenum-graecum*	4.4	7.3
45	*Origanum marjorana*	4.5	7.3
46	*Rosmarinus officinalis*	4.5	7.4
47	*Salvia officinalis*	4.5	7.5
48	*Thymus zygis*	4.7	7.8
49	*Citrus aurantium bergamia*	4.8	7.8
50	*Citrus sinensis*	4.8	7.8
51	*Cinnamomum spp*	4.9	7.8
52	*Thymus vulgaris*	4.9	8.1
53	*Vitex negundo*	5.1	8.2
54	*Cymbopogon citratus*	5.3	9.0
55	*Mentha arvensis*	5.4	8.8
56	*Origanum compactum*	5.6	9.2
57	*Murayya koenigi*	5.7	9.3
58	*Syzygium aromaticum*	6.0	9.6
59	*Para-Menthane-3, 8-diol*	6.3	10.3
60	*Cannabis sativa*	6.3	10.3
61	*Ferrula gummosa*	6.6	11.2
62	*Satureja hortensis*	7.2	11.3
63	*Artimisia pallens*	7.6	11.9
64	*Lavandula latifolia*	7.6	11.9

65	*Thymus mastichina*	7.7	12.8
66	*Lavandula hybrida variety*	7.8	13.0
67	*Myristica frangrans*	8.5	13.7
68	*Pelargonium graveolens*	9.3	14.6
69	*Satureja montana*	12.6	19.7

3. Results

Table 2: Top eight essential oil moieties having promising mosquito knock down potential arranged by KT_{50} values in ascending order.

Sr. No.	Items	Family	KT_{50} (min.)	KT_{95} (min.)
1	Market Anti-Mosquito coil (0.1% Esbiothrin)_ Standard	NA	1.9	3.5
2	*Tanacetum annuum*	Asteraceae	1.9	3.5
3	*Pogostemon cablin*	Lamiaceae	2.0	3.9
4	*Copaifera langsdorffii*	Fabaceae	2.1	3.7
5	*Cuminum cyminum*	Apiaceae	2.1	3.7
6	*Cedrus deodara*	Cupressaceae	2.4	4.0
7	*Achillea millefolium.*	Asteraceae	2.7	5.1
8	*Artemisia absinthium*	Asteraceae	2.7	5.1
9	*Helianthus annus*	Asteraceae	2.7	5.1

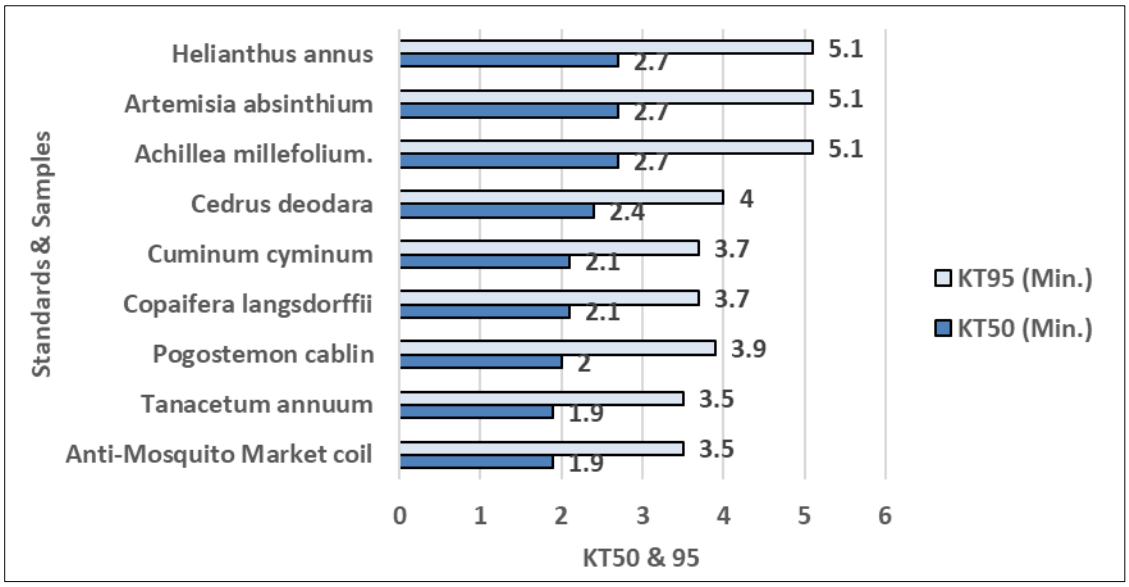


Fig 2: Promising Anti-mosquito Moieties

4. Discussion

Finding novel active moieties for developing new innovative products with unique selling preposition is the need of the hour in Fast Moving Consumer Goods market. Therefore, the objective of the current novel method is to shortlist promising candidates from the ocean of botanicals and that is the first step towards developing botanical insecticidal household products. In industrial set up, time is the key factor i.e. how fast the things are delivered matters a lot. Current quick assay requires less manpower and less mosquitoes, besides saving time and cost.

Usefulness and credibility of the method is proved by finding Lamiaceae oil as prominent herbal active moiety which got translated in anti-mosquito incense stick product that gives mosquito protection to consumers.

On the contrary, there is other method of screening the active that involves one square meter Peet Grady Chamber ($1M^3$). This method also requires huge time i.e. 180 minutes for each experiment run involving actual experimental evaluation (1hour), extra man hour for cleaning and drying the glass chamber (1 hour), 1 hour for blank experiment for contamination check. Moreover, it also needs many mosquitoes for complete run and maximum of three experiments i.e. only one moieties can be evaluated daily.

Therefore, newly devised method is proved beneficial for screening hundreds of moieties in a less period of time.

However, current assay also has got a limitation that basis outcome of the results the efficacy of respective moiety is not confirmed. For further confirmation, the mosquitocidal evaluation has to be done in Peet Grady Chamber ($5.83M^3$) against larger mosquito population [7, 8].

5. Conclusion

The novel method devised is beneficial since it saves time and energy. Shortlisted candidates were again evaluated in Peet Grady Chamber ($5.83M^3$) with larger female *Aedes* mosquitoes, subsequently, promising moieties can be translated in product development followed by market launch. By following this newly developed process, shortlisted ingredients were translated in development of incense stick that gives protection from mosquitoes post evaluations in peet grady chamber ($5.83M^3$) [7, 8]. Thus, we have enough confidence on method precision and robustness.

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