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Research Article

NEW INTERVENTIONS IN FUMIGATING WITH *APARAJITHA DHOOMA CHOORNAM*

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KEYWORDS: Microbicidal, fumigation, Ayurvedic incense sticks, insecticidal, bioassay.

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ABSTRACT

Aparajitha dhooma choornam is a traditional Ayurvedic medicine used to disinfect the environment. The microbicidal properties of Ayurvedic *Dhoopana* have been previously documented. The safety and efficacy of herbal products can be improved by incorporating modern technology while fumigating. The present study aims to show the efficacy of fumigation with *Aparajitha dhooma choornam* sticks in reducing microbial flora of work spaces so that it can be used conveniently on a regular basis to improve air quality and reduce the incidence of spread of airborne diseases. Bioassay studies were also carried out to check the insecticidal activity of *Dhoopana* against both larvae and adult mosquitoes found at site. Thus fumigation with *Aparajitha dhooma choornam* sticks and powder form was found to be effective in not only reducing microbial load but also in possessing insecticidal activity.

INTRODUCTION

The *Kashyap Samhita* gives detailed procedures for fumigation under the chapter *Dhoomakalpadhyaya. Dhupana* is said to be very helpful to prevent diseases by disinfection of desired places. Various herbs are used in fumigation. Binding agent and aroma can be added for pleasant smell along with effective action. *Janapadodhvamsa* of *Caraka samhita* and *Sushrutha Samhita* prescribe fumigation for disinfection and treatment.[1] Traditionally fumigation is carried out by making hot embers with cow dung cakes or coconut shells and then putting the powder on the embers. The fumes which emanate can be used to disinfect rooms and the surroundings. It is purported to control pests, rodents and insects and helps prevent outbreaks of contagious diseases. It can be used safely as it does not have the toxicity of chemical fumigants.[2] *Aparajitha dhooma choornam* is one such formulation mentioned in classical Ayurvedic texts which is very effectively used for fumigation. But, the powder form is cumbersome to use and most modern dwellings do not have the wherewithal to make burning embers for fume production. Also burning embers may become a fire hazard in closed working spaces if left unmonitored. Conversely, the stick forms are easy to use and when encased in earthen or metal containers need less supervision. Thus there is a need to develop safer alternative methods which are convenient to use.[3] Hence, *Aparajitha dhooma choornam* was converted to sticks form. The present study focussed on comparing the efficacy of powder as well as stick forms of fumigation by assessing the effect of the fumes on air microflora and also their effect on larval and adult mosquitoes. The mortality of larvae and adult mosquitoes of public health importance were scored and their repellent effects studied. As per our knowledge, this is the first study investigating the insecticidal activity of *Aparajitha dhooma choornam* along with its antimicrobial effect. These studies are significant given the emergence of new diseases and the phenomena of insecticide resistance. Tapping alternative resources like the use of *Aparajitha dhooma choornam* is the need of the hour and the present study aims to give a comprehensive investigation on its efficacy on microbes and mosquitoes. Here we have tested the fumes both on larvae as well as adult mosquitoes as we found that there is a paucity of work on toxicity against adult mosquitoes and many studies against larvae as they are comparatively easier to work with than adult mosquitoes.

MATERIALS AND METHODS

1. Preparation of *Aparajitha dhooma choornam* Incense Sticks

The different components used for the preparation of the *Aparajitha dhooma choornam* incense sticks were as follows:

Table 1: Ingredients used to prepare Aparajitha dhooma choornam incense sticks

S.No.	Name of Ingredients	Botanical name	Plant part used
1	*Gulgulu*	*Commiphora mukul*	Gum resin
2	*Vayambu*	*Acorus calamus*	rhizome
3	*Chenchalyam*	*Shorea robusta*	resin
4	*Aryaveppin tholi*	*Azadirachta indica*	bark
5	*Erukkinveru*	*Calotropis gigantea*	root
6	*Karakil*	*Aquilaria agallocha*	Heart wood
7	*Devatharam*	*Cedrus deodara*	Heart wood
8	*Karutha Katuku*	*Brassica nigra*	seed
9	*Jigath*	*Litsea glutinosa*	Bark powder
10	Saw dust	*Aquilaria agallocha*	Bark powder
11	camphor	*Cinnamomum camphora*	resin
12	Bamboo *sticik*	*Bambusa vulgaris*	Stem sticks

Gulgulu was taken in a hot pan and roasted. After cooling, it was mixed with other ingredients excepting *Jigath*, saw dust and camphor. This mixture was then pulverised, sieved and collected as a fine powder. This powder was then mixed with *Jigath*, saw dust powder and camphor powder. Water was added to make dough mass. The paste was then coated onto the sticks semi automatically and then dried in a hot air oven for 2 days at 40^0C and then stored in air tight plastic bags.[4] Till now there were no quality specifications for incense sticks but the Agarbattis - Specification (Tentative Standard) brought out by Bureau of Indian Standards (BIS) - IS 13582 is a step in the right direction. This will help improve product quality and enable value addition to products.[5]

2. Comparison of Anti-microbial Action Using *Aparajitha dhooma choornam* Powder and Incense Sticks.

The study of air quality before and after fumigation was carried out in Packing unit and Research and Development formulation unit. It mainly focussed on the antimicrobial effects of *Aparajitha dhooma choornam* by sedimentation plate technique using sterile Nutrient agar plates and Sabouraud's agar plates.

In the present study, comparison of fumigation using conventional *Aparajitha dhooma choornam* powder and *Aparajitha dhooma choornam* sticks was carried out.

Fig 1: Research and Development formulation unit *Aparajitha dhooma choornam* sticks were lit

Fig 2: Packing unit, *Aparajitha dhooma choornam* powder was smoked

The sticks were lit in Research and Development formulation unit (approximately area 45 X 35 X 25 sq.ft) and *Aparajitha dhooma choornam* powder was smoked in packing unit (approx.. area 40 X 30 X 25 sq.ft). Since it was inferred that the proper correction should be made to equalize the concentration of *Aparajitha dhooma choornam* present in the sticks with that of powder alone. Therefore, weight correction was applied as follows:

Weight of stick 0.2 gms

Prepared stick 0.9 gms

% of *Aparajitha dhooma choornam* powder in combination 43%

Weight of *Aparajitha dhooma choornam* powder in 150 gms - 50 gms.

Hence, for 100gms of powder, 300 gms of sticks needed to be taken.

Since these are industrial units, 350 grams of sticks in Research and Development formulation unit and 100 grams of *Aparajitha dhooma choornam* powder in packing unit were respectively weighed and used. This was to adjust the size difference in the rooms. *Aparajitha dhooma choornam* powder was added to hot embers and smoke produced whereas sticks were lit to produce smoke. Fumigation was carried out after the rooms were empty of personnel and fumes exposure continued for 1 hour. Afterwards, the rooms were opened and exhaust fans put on to remove traces of smoke. The nutrient agar and Sabouraud's agar plates for sedimentation plate method was kept for a ten minute exposure period before fumigation. After 1 hour of fumigation and later ventilation, sedimentation plate technique was carried out with nutrient agar and Sabouraud's agar plates to observe the effect after fumigation. Comparing the physical nature, similar fumes were observed and the burning efficiency for *Aparajitha dhooma choornam* incense sticks to burn completely was found to be one hour.

3. Mosquito Collection, Rearing and Identification

Mosquito collections were made from their natural breeding habitats from gardening areas as well as outdoor bushes. Sampling of larvae was made from rainwater pools and empty pots. The larvae were reared in a temperature-controlled space at 25 ± 5°C and 80% ± 10% relative humidity so that both larvae and adults could be assayed in the laboratory of Research and Development Centre, Oushadhi.

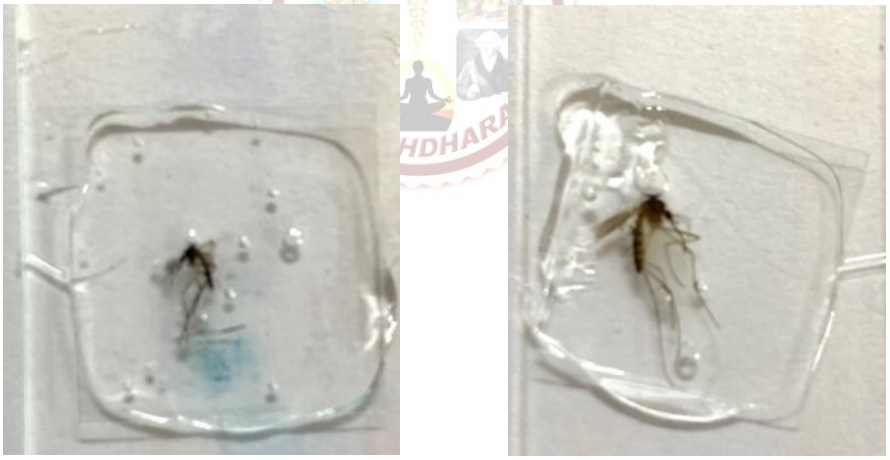


Fig 3: Mosquitoes which were obtained during knockout were fixed using DPX mountant and then later viewed under the Research trinocular microscope for identification characteristics.

4. Assessment of Larvicidal Activity

To evaluate the biological activity of a mosquito larvicide, laboratory-reared mosquito larvae of known age or instar (F1 of field-collected mosquitoes) were exposed for different times to the fumes of *Aparajitha dhooma choornam*, both sticks and powder. Homogenous populations of mosquito larvae or given instar were obtained using standardized rearing methods.[6]

Batches of 25 third or fourth instar larvae were transferred by means of fine brushes to test tubes each containing 10–15ml of water. Small, unhealthy or damaged larvae were removed and replaced with healthy ones. The depth of the water in test tubes was kept between 5cm and 10cm as deeper levels have been known to cause undue mortality.[7]

After different time periods of exposure, larval mortality was recorded. Exposure for larvae was set up in glass solvent chamber as follows:

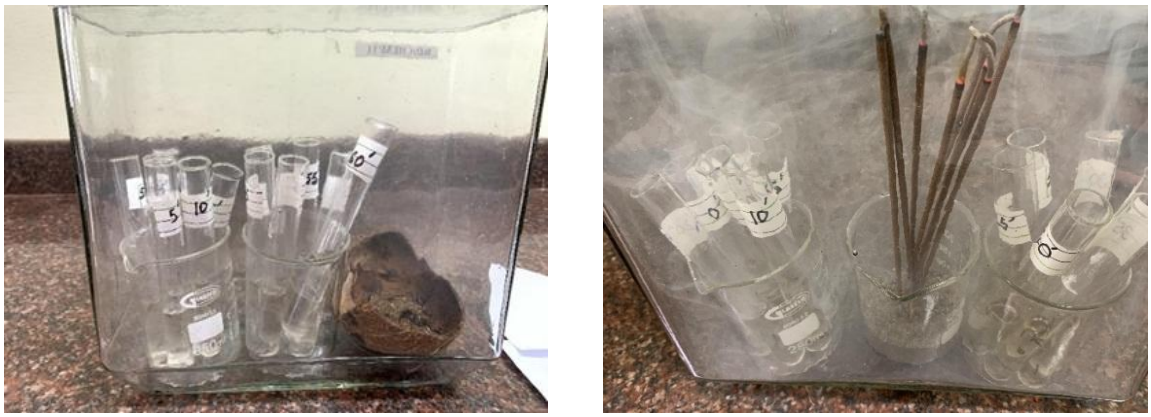


Fig 4: Solvent chamber set up with *Aparajitha dhooma choornam* powder and sticks

Fig 5: The test tubes with larvae for exposure studies. Larvae exposed for 0, 5 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 minute intervals.

Moribund larvae were those unable to rise to water surface or show diving action on disturbing water. These were also counted along with dead larvae so as to calculate percentage mortality. Bioassays were done in triplicate and bioassay results reported.

5. Assessment of Activity Against Adult Mosquitoes

Exposure of adult mosquitoes was done using a modified fume hood set up. *Aparajitha dhooma choornam* powder or sticks were lit and the fumes allowed to fill in the fume hood. This was somewhat similar to the method used by Jaswanth et al. [9] 20-25 adult mosquitoes were placed in plastic bags with holes small enough to prevent escape of mosquitoes but large enough to allow entry of gases. Knockdown score was taken by counting the mosquitoes that were unable to fly or were on their backs. Knock-down times were determined by visual analysis.

Fig 6: Modified mosquito bioassay chamber -Fume hood filled with smoke after fumigation with *Aparajitha dhooma choornam*

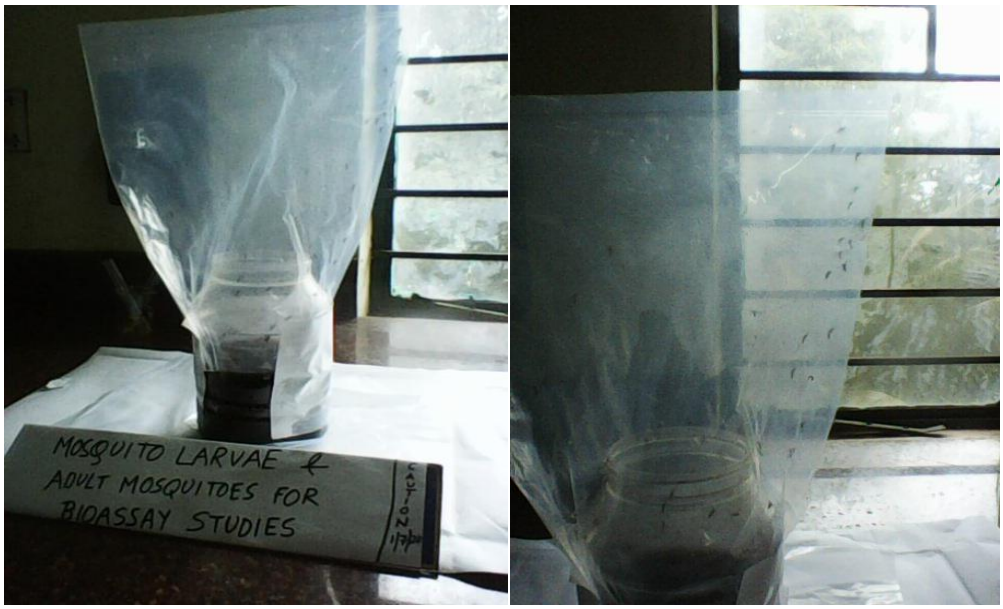


Fig 7: Mosquitoes trapped in polythene bag for bioassay

RESULTS AND DISCUSSION

I. The incense sticks thus formulated were checked for their quality using features like surface evenness and smoothness. The average weight of the incense was fixed at 1.5 gm with a thickness of 0.5 cm. Loss on drying was kept at < 5%. The final product had a creamish to dark brown colour and the incense sticks were uniform in shape.

II. Sedimentation Exposure Plate Results

The sedimentation exposure plates were read after incubation and their total plate count and yeast and mould plate count values were evaluated. [10]

Table II: Effect of fumigation of microbial air quality of industrial enclosed spaces. Counts before and after fumigation

Section / Microbes tested	Packing – Mean cfu/m³		Formulation- Mean cfu/m³	
	Before	After	Before	After
Standard Plate Count	24±7.51	11.67±1.37	37.67±6.5	13.33±2.34
Yeast and mould	9.33±1.63	3.83±0.75	9±0.89	4.33±2.34

Table III. Percentage reduction in microflora using the two fumigation methods in packing unit and R & D Formulation unit after applying weight correction

Experimental area.	Bacterial counts	Yeast and mould counts.
% reduction in Formulation area (using Powder)	55.54%	41%
% reduction in Packing unit (using stick)	48.62%	48.11%

The efficiency of the *Aparajitha dhooma choornam* sticks was found to be comparable to *Aparajitha dhooma choornam* powder.

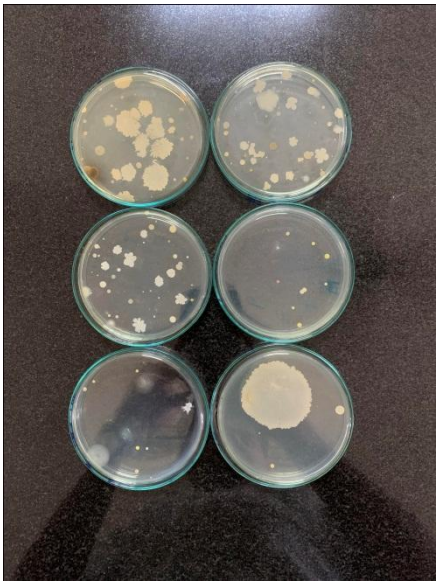
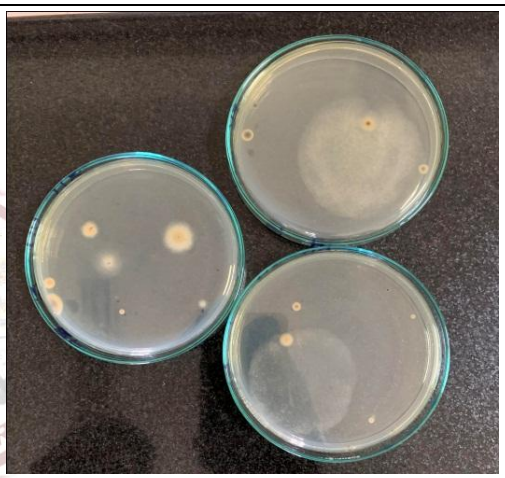
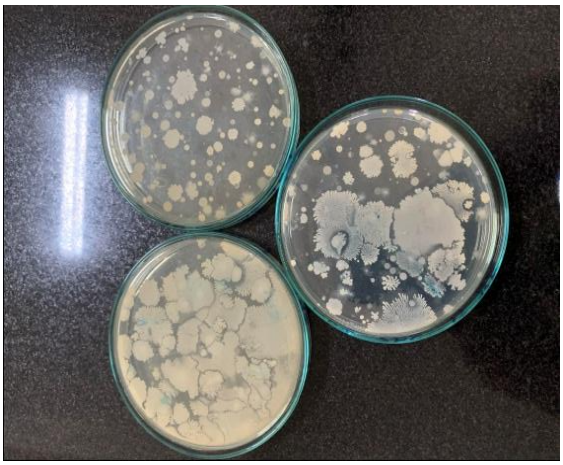
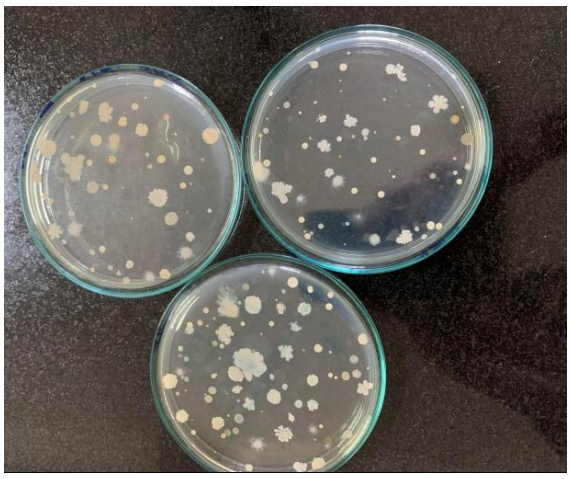
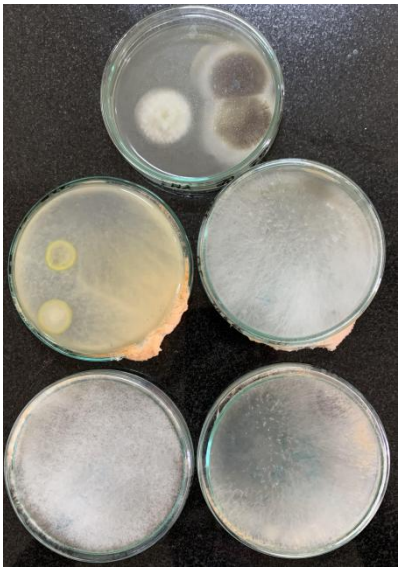
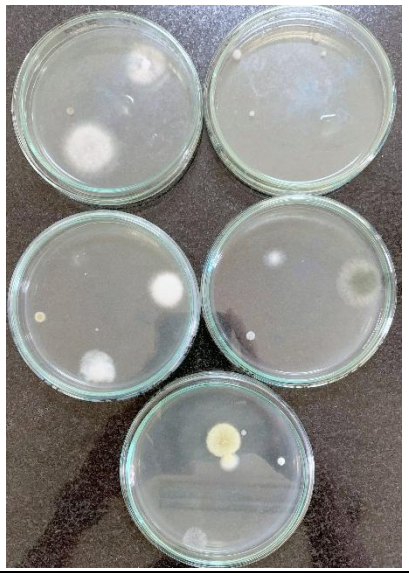
	
SPC plates before fumigation	SPC plates after fumigation
	
Yeast and mould cfu/m^3 before fumigation	Yeast and mould cfu/m^3 after fumigation
	
Bacterial cfu/m^3 before fumigation	Bacterial cfu/m^3 after fumigation

Plate exposure before fumigation	Plate exposure after fumigation
	
Yeast and mould plates before fumigation	Yeast and mould plates after fumigation

III. Mosquito collection, rearing and identification

The larvae and pupae collected from the garden sites at Oushadhi were kept in the laboratory for getting sufficient adult population for bioassay studies. The garden caught mosquitoes and emerged adults were morphologically identified to species level by entering the various key parameters for identification. Identification was based mainly on adult characters. Specimens were identified as adults using the morphology-based keys of the Walter Reed Biosystematics Unit.[11,12,13.]

They were identified peremptorily as *Aedes kochi* and *Culex* spp based on their morphology based keys after noting the characteristics using binocular microscopy images of the mounted specimens.

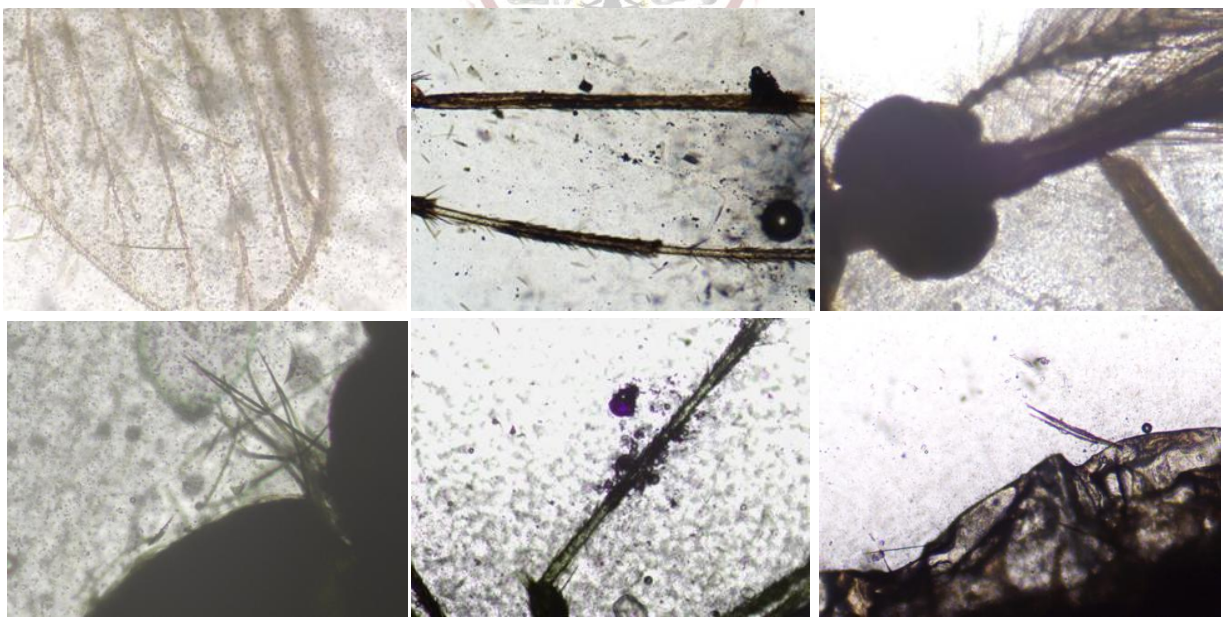


Fig 8: Based on head, thorax, wing and leg characteristics as seen under Microscopic images taken at 40 X and 100X, Magnus Olympus microscope

IV. Assessment of Larvicidal Activity

First trial with *Aparajitha dhooma choornam* powder, not so successful and so cumbersome to use. More than 1 hour to observe first larval knockout. Second trial with *Aparajitha dhooma choornam* sticks. User friendly and application led to knockout of one larvae each at 45 mins and 50 mins respectively. At 60 mins, 4 out of 5 larvae were knocked out.Mosquito adults were also exposed to *Aparajitha dhooma choornam* fumes

and >90% knockout seen within 20 mins of application. Arrangement of larvae for bioassay was with exposure times 0 mins, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 mins. Mosquito larvae were scored for death or as moribund larvae in the tubes.[14]

V. Assessment of Activity Against Adult Mosquitoes

Trials	***Aparajitha dhooma choornam* powder Knockout time in mins**		***Aparajitha dhooma choornam* sticksKnockout time in mins**	
	Aedes kochi	***Culex* spp**	***Aedes kochi***	***Culex* spp**
Trial 1	45 mins	30 mins	21 mins	19mins
Trial 2	40 mins	35 mins	20 mins	20 mins
Trial 3	35 mins	35 mins	21 mins	20mins
Mean values	40 mins	33.3 mins	20.6 mins	19.6 mins

It is seen that there is greater efficacy with *Aparajitha dhooma choornam* sticks as compared to *Aparajitha dhooma choornam* powder. This may be due to the fact that there is fume reduction once powder is exhausted, but the incense sticks continue burning.

Conclusion and Application Translatable to Industry

This work gives a scientific validation to fumigation using *Aparajitha dhooma choornam*. It also supports the use of sticks which is more customer friendly and gives comparable results after weight correction is applied.

Since we tried it in industrial premises, more sticks were used. However in homes, less sticks can give appreciable results and help in controlling air microflora.

Aparajitha dhooma choornam sticks showed more good results in the control of bacterial colonies than fungal propagules. The monsoon season as well as the ongoing Covid 19 pandemic has made people aware of good sanitation practices and air hygiene. People are more prone to contagious diseases caused by microbes during the monsoon. Fumigation with *Aparajitha dhooma choornam* in homes can enable disinfection and be an important preventive measure. *Aparajitha dhooma choornam* sticks have numerous benefits over powder:

1. **Ease of handling:** The sticks can be marketed in packets like normal Agarbattis.
2. **Eco friendly:** Since they are made from 100% ecologically degradable materials they are environment friendly and do not contribute to smoke pollution as burning powder over charcoal does.
3. **Ease of portability:** They can be carried around in a safe manner and applied even in outdoor settings. They can be used at home as well as carried outdoors for use.
4. **Lasts longer:** Burning time is approximately one hour. The sticks can be lit by a direct flame and does not need continued application of heat from burning embers in earthen receptacles. An agarbatti holder is sufficient and the nice fragrance can make it a dual source for both religious and home purification purpose.
5. **Odor free and no charring:** Ease of application leads to an odor-free environment in public places and can be effectively used in hospitals, hotels, offices and industries with very little supervision and better safety profile as compared to powder fumes. Burning embers for powder fumigation can be dangerous in certain workplaces if left unattended. But sticks in enclosed stick holders have better safety.
6. **Ease of application:** In modern homes; people do not have the wherewithal to make burning embers for the formation of fumes from *Aparajitha dhooma choornam* powder. For such settings, sticks can be more conveniently used.

Hence it is suggested that *Aparajitha dhooma choornam* sticks may be preferred over powder and it be produced and marketed for better outreach and customer satisfaction. This will be a yeoman service to society especially in the wake of the pandemic so that the stick use becomes more prevalent and helps in maintenance of air hygiene.

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