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Assessment of efficiency of mosquito repellents using different natural plant extracts

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Abstract

Mosquitoes transmit a range of infectious agents that affects human health, Malaria, dengue fever, yellow fever and other mosquito-borne disease infect countless individuals and accounts for thousands of deaths annually, causing a large burden to public health and on the economic process of developing countries. At present, concerning 40% of the world's populations mainly in India live in areas where the chance of dengue fever transmission is most.

Controlling such diseases is extremely difficult due to the higher reproduction rate and development of mosquitoes that are resistant to insecticides and commercially available repellents. Chemical mosquito repellents contain toxic synthetic pyrethroids as active ingredients whose exposure to food and water is hazardous to health. Cow dung places a major role since time immemorial and it is considered as a third important services rendered by animals. Dung is utilized as bio fertilizer, cheap fuel and insect repellent.

The Cow dung is waste material and completely free. It is great business strategy to use the waste in to a valuable product. Many plants have evolved aromas that repel mosquitoes and other herbivores. In present study, an attempt has been made to develop an eco-friendly dhoop sticks containing Cow urine (Gomutra), cow dung, Plant material: Leaves of Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), Rui (*Calotropis gigantea*), Durva grass (*Cynodon dactylon*), Ashoka (*Saraca asoca*), by using this material, 'dhoop stick', 'extract cards' etc. were prepared and their anti-mosquito activity was determined. The burning of these aids not only controlled mosquitoes but also reduced air micro flora from surrounding area.

Keywords: Disease, mosquito repellent, plant extract, cow dung, mosquito stick', 'extract cards'

Introduction

Mosquito borne disease are major human-health problem in all tropical and subtropical countries. The disease transmitted include malaria, filariasis, yellow fever, Japanese encephalitis and dengue fever *Culex quinquefasciatus*, the potential vector of lymphatic filariasis, is the most widely distributed tropical disease with around 120 million people infected worldwide and 44 million people having common chronic manifestation ^[1]. Controls of such serious diseases are becoming increasingly difficult because of high rate of reproduction and development of resistance to insecticides in mosquitoes ^[2]. Several vector control measures such as biological, chemical, environmental and individual protection measures are taken to prevent spread of malaria, dengue fever and other mosquito-borne diseases. Personal protection is one of the established methods to prevent mosquito bites. Insect repellents date back to ancient times, with the use of tars, smokes, plant oils and other modalities ^[3]. In recent years, new synthetic repellents have been formulated and advocated. However, constant and indiscriminate use of these synthetic repellents causes adverse effects on the user ^[4]. Many plants have evolved aromas that repel mosquitoes and other herbivores ^[5]. Plant cow dung based products have been used as repellents either as topical applicant or fumigant and many plant species showed mosquito repellent and insecticidal property ^[6]. There are numbers of health associated problems developed by constant use of synthetic mosquito repellents. To solve these problems, in present work, anti-mosquito products of plant cow dung origin are being rejuvenated. The leaf extracts of Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), Rui (*Calotropis gigantea*), Durva grass (*Cynodon dactylon*), Ashoka (*Saraca asoca*) and additional to this Cow urine (Gomutra), cow dung, Neem oil, distilled water, and Whatmann filter paper-1 were used. By using this material, 'dhoop stick', 'extract cards' etc. were prepared and their anti-mosquito activity was determined.

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Materials and Methods

Plant material: Leaves of Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), Rui (*Calotropis gigantea*), Durva grass (*Cynodon dactylon*), Ashoka (*Saraca asoca*). Additional material: Cow urine, Cow dung, Neem oil, Distilled Water, Wax and Whatmann filter paper-1 was used for the experiment.

Preparation of Dhoop sticks

Dhoop sticks were prepared by using fresh Indian cow dung and leaves extract of each selected plant separately. For this preparation, 5 gm. of freshly collected Indian cow dung were added in 5 ml of each plant extract separately. One important thing should be kept in mind that Indian cow dung and urine gives better results as compared to other species. After preparation of sticks allow it for sun drying. Then, use completely dried sticks for further experiment.



Fig 1: Dhoop sticks

Preparation of extract

Five gram of plant leaves were collected and rinsed in distilled water. By using mortar and pestle, these leaves were crushed using distilled water and cow urine with 1:1 proportion separately. This way two types of extract were prepared, one in distilled water and another set was in cow urine (Gomutra). These extracts were used for further preparations of mosquito repellent aids.

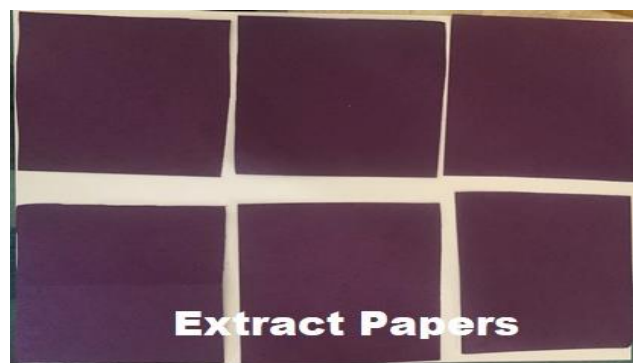


Fig 2: Extract papers

Preparation of Extract Paper

Extract papers were prepared by using Whatmann filter paper-no.1. These papers were soaked in each extract and dried it to prepare extract Paper.

Along with these experimental aids, one set of control aid were also prepared. For example, control dhoop stick was

prepared by adding only distilled water instead of leaves extract, control Paper was prepared by soaking Whatmann filter paper in distilled water and in only cow urine.

Collection of Mosquitoes

Mosquitoes were collected from surrounding area of college campus Daushree Vashudev Chandrakar Kamdhenu Visvavidyalaya Anjora durg and public places. Collected mosquitoes were of different species. Mixed population of mosquitoes were used for further experimentation. Anti-mosquito activity (mosquito repellent activity): Mosquito repellent activity was checked by using 10 mosquitoes (irrespective of their species) per exposure to each extract aids smoke (Dhoop sticks, extract paper) in closed cabinet. Area of experimental cabinets was 3.5ft X 2ft X 4ft. The cabinet had a small opening at one side. That opening was closed by net at initial period of experiment. During this exposure behavior (like gathering of mosquitoes toward net area for escaping) of mosquitoes were recorded. After one hour, for checking mosquito repellent activity, experimental cabinet was opened to form a small opening. The mosquitoes that successfully ran away, also counted to determine repellent efficiency of aids.

Antimicrobial activity

For checking antimicrobial activity Nutrient agar plates were prepared. Before and after burning of all extract Paper; air micro flora of exposed area were checked by exposing NA plates to respective area for one minute each. Nutrient Agar plates were exposed to check normal micro flora of selected area. This plate is labelled as negative control. After exposure, plates were kept in incubator for 24 hrs. For checking antimicrobial activity of each extract paper, the burning extract paper was kept in selected experimental cabinet. After complete burning of paper, NA plate was exposed to air of that cabinet for 1 minute. These plates were labelled with respective plant and extract name. All plates were kept at 37 °C in incubator for 24 hrs.

Results

In the present study, the mosquito repellent activity of selected local plants extract with cow dung and cow urine were evaluated. After complete burning of Dhoop stick or Extract Paper total remaining mosquitoes were counted.

In case of Dhoop sticks Prepared from Neem (*Azadirachta indica*), Rui (*Calotropis gigantea*), Durva grass (*Cynodon dactylon*), Asoka (*Saraca asoca*) show their repellent action against Mosquitoes in descending order Tulsi (9%) > Neem (5%) > Durva (4%) > Rui (4%) > Ashoka (2%).

Due to closed experimental area, few mosquitoes get killed and few of them became unconscious. After opening of cabinet, few mosquitoes escaped from that area. The comparative data of anti-mosquito activity showed that, effect of dhoop sticks burning was more than Extract Paper and in case of all plant extract Tulsi (7%) > Neem (4%) > Durva (5%) > Rui (3%) > Ashoka (1%). (Table No.1).

The comparative study of effect of plant extracts showed that, extract of Tulsi (*Ocimum sanctum*) that is prepared in cow urine was more effective than distilled water extract.

Effect of smoke on air micro flora

After 24 hrs of incubation, microbial growth on each plate was recorded. The negative control plate showed full growth but plates that were exposed to extract paper smoke showed

only few microbial colonies. The experiment showed anti-microbial potency of different plant extract paper smoke in descending order which is given below, Tulsi> Mixed extract >Neem>Durva>Rui>Ashoka.

Based on these observations, it is clear that the burning of dhoop sticks not only reduces mosquito population but also helps to reduce general air micro flora (Table No.1).

Discussion

Plant products can be used, either as an insecticide for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess [7]. Smoke is still the most widely used common method of repelling biting insects that is used throughout the world. Fresh and dried plants are frequently added to fires to enhance the repellent properties of the smoke. Several field evaluations, where plants were burned to repel mosquitoes have shown good reduction in mosquito landings [8]

In present investigation, mosquito repellents aids like Dhoop sticks and Extract card, were prepared by using traditionally local plants, Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), Rui (*Calotropis gigantea*), Durva grass (*Cynodon*

dactylon), Ashoka (*Saraca asoca*) and additional to this Cow urine and cow dung were used.

Amongst all the plants, Tulsi (*Ocimum sanctum*) and mix extract paper showed highest mosquito repellent activity. The experimental results showed anti mosquito activity in descending order of Tulsi>Mix extract >Neem>Durva>Rui>Ashoka. These results were found in all aids prepared using plant materials. From the two aids, dhoop stick showed maximum mosquito repellent activity than ‘Extract paper’. The aids prepared from plant extract are best option for chemically synthesized mosquito repellent. The ingredients of cow urine and phytochemical compounds of plant extract are responsible for mosquito repellence.

The mosquito coils available in market creates heavy smoke that can causes severe respiratory problems especially for patients of Asthma. Though the ‘Fast card mosquito papers’ are available in market, they contain of TFT molecules which has adverse effect on respiratory system. By repeated use of mosquito coils and fast card paper, mosquitoes may acquire resistance against smoke and TFT molecules. These problems can be reduced by using plant extract dhoop stick with more efficiency for longer time.

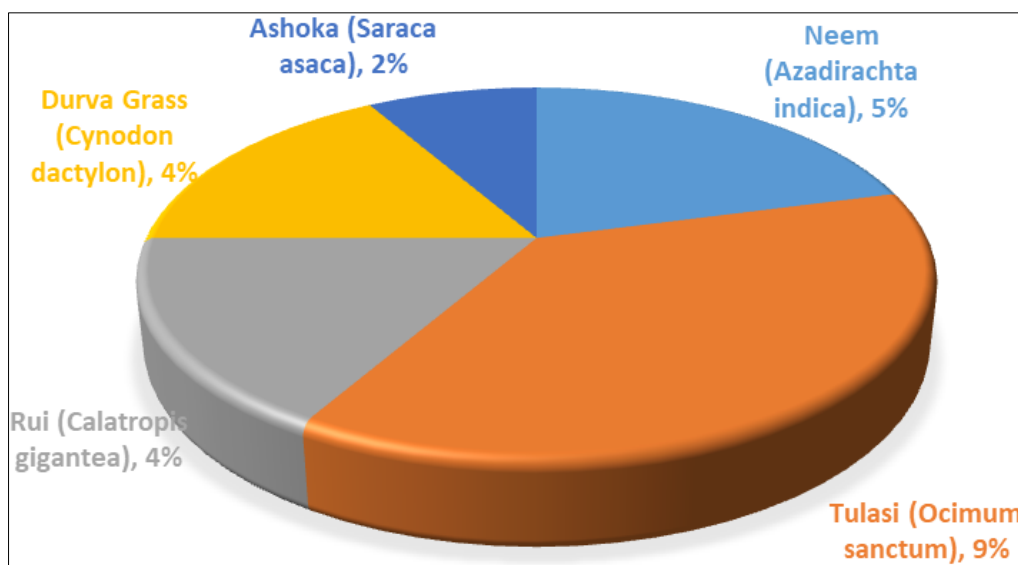


Fig 3: Show the Neem and Ashoka different

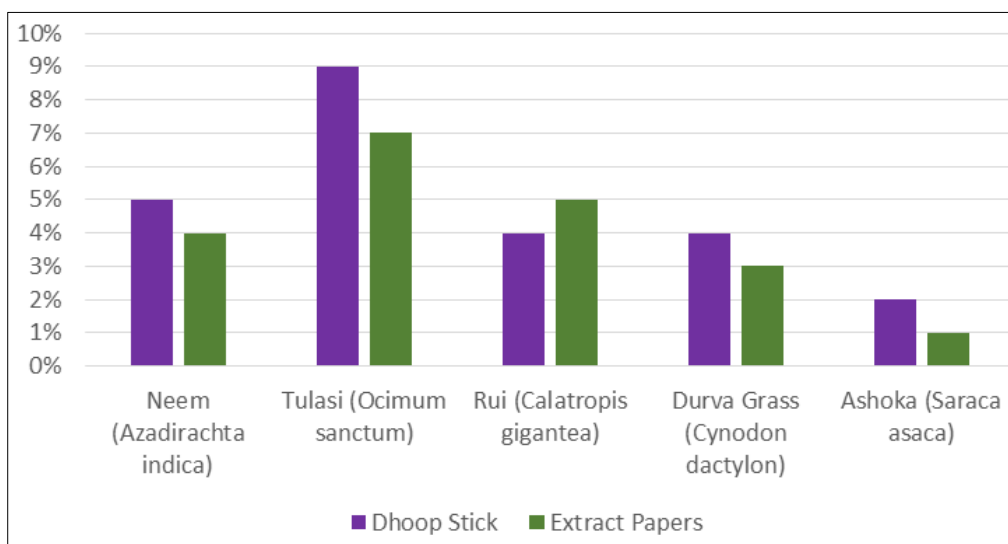


Fig 4: Effect of dhoop stick and paper extract on mosquitoes

Table 1: Effect of Dhoop Stick and paper extract on Mosquitoes

Plant Powder	Dhoop Stick	Extract Papers
Tulsi (<i>Ocimum sanctum</i>)	9%	7%
Neem (<i>Azadirachta indica</i>)	5%	4%
Rui (<i>Calatropisgigantea</i>)	4%	5%
Durva Grass (<i>Cynodon dactylon</i>)	4%	3%
Ashoka (<i>Saraca asaca</i>)	2%	1%

Conclusion

The developed herbal mosquito repellent provide long lasting protection, safe for human life, human and domestic animal skin with no side effect and no feedback of environmental ill effect, as an alternative to synthetic chemical repellents. The raw materials have been selected based on experience and practice by ancestors. The formulation is safe, eco-friendly, cheap, easy to use and has maximum repellence against mosquitoes. In addition this, the mosquito repellent is less harmful to our health than the ones available in the market.

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