



Development and evaluation of low-cost mosquito repellents using natural binders

Selchi Devi^{1*}, Madhubanti Das¹, Bhagyashree Mahanta², Dhananjay Kusre¹

¹ Assistant Professor, Department of Zoology, Morigaon College, Morigaon, Assam, India

² Department of Zoology, University of Science and Technology, Meghalaya, India

Abstract

Mosquito-borne diseases pose a significant threat to global health, emphasizing the need for effective and accessible mosquito repellents. This study aimed to develop and evaluate two innovative, low-cost mosquito repellents using natural binders. The first repellent was a spray formulation prepared using neem leaves (*Azadirachta indica*) and marigold flowers (*Tagetes erecta*) extracted through Soxhlet extraction. The second repellent was a cow dung cake mixed with neem leaves paste, leveraging the natural binding properties of cow dung. Both repellents were evaluated for their efficacy through rear mosquito tests, cage tests, and phytochemical screening. pH tests were also conducted to assess the stability of the formulations. Results showed that both repellents exhibited significant mosquito repellency. Phytochemical screening revealed the presence of bioactive compounds, including azadirachtin and lutein, contributing to the repellents' efficacy. The cow dung cake mixture showed a unique slow-release property, prolonging of natural binders in creating effective, low-cost mosquito repellents, offering a sustainable alternative to synthetic products. Further research can optimize and scale up production for community use.

Keywords: Mosquito repellent, low-cost, eco-friendly, soxhlet extraction

Introduction

The World Health Organization (WHO) estimates that mosquito-borne diseases cause over one million deaths worldwide each year. Mosquito-borne diseases, such as malaria, dengue fever, and Zika virus, pose significant public health threats globally, particularly in tropical and subtropical regions (Sukumar *et al.*, 1991) [2]. Synthetic mosquito repellents, such as DEET and picaridin, are effective but have raised concerns about environmental pollution, skin irritation, and potential neurotoxicity. In contrast, natural mosquito repellents offer a safer and more sustainable solution (Pandey *et al.*, 2011) [9].

Neem leaves and marigold flowers have been used in traditional medicine for centuries, and their insecticidal and repellent properties have been extensively studied. Neem contains compounds like azadirachtin, which disrupts mosquito hormone systems, while marigold contains pyrethrum, a natural insecticide. Cow dung cakes have also been used in some regions as a natural insect repellent, possibly due to the presence of volatile organic compounds (Kothari *et al.*, 2011) [11].

Despite the potential of these natural materials, there is a need for systematic evaluation and development of effective mosquito repellent formulations. This study addresses this gap by developing and testing two natural repellents using neem leaves, marigold flowers, and cow dung cake, with the goal of providing a low-cost, sustainable solution for mosquito-borne disease compounds.

Materials and methods

Collection of plant materials

The neem leaves and marigold flowers were collected from Morigaon district in the month of April 2024 (26.2600° N, 92.2630° E). The parts of the plants that were segregated were washed thoroughly with tap water. The plant extract

was then prepared using a suitable standard protocol for further use.

Preparation of plant extract

The neem leaves and marigold flowers were dried and made into a coarse powder individually using a mortar and pestle. The active ingredient in plants was extracted from coarse powder by using a Soxhlet extractor.

Mosquitoes rearing

The mosquitoes were reared and maintained in the laboratory, Department of Zoology, Morigaon College, following the standard rearing procedure (Morlan *et al.*, 1963; Promsiri *et al.*, 2006). The mosquitoes used in this experiment were caught using a net while biting humans between 6 p.m. and 9 p.m. 25 mosquitoes were placed in the cage. The adult females were allowed to take a blood meal on albino rats. The rat was confined to a cage to restrict its movement. A filter paper strip was wrapped inside a glass beaker containing almost half-filled water and placed inside the cage for egg-laying.

Culture of *Aedes aegypti* larval stages

Aedes aegypti larvae were cultured in the laboratory at Morigaon College and maintained in the laboratory. 60-70% relative humidity and a photoperiod of 14:10 h (light: dark) during all experiments. The larvae were fed with yeast extract the first day after eclosion and later with ground dog biscuits.

Preparation of mosquito repellent products

Natural spray for mosquito repellent

The herbal repellent spray was prepared by infusion as well as nano-emulsion. We added the required amount of

distilled water to the beaker, heated it, and infused it. In addition, the essential oils and other substances were infused to form a milky substance (Nano Emulsion). Finally, the mixture was cooled to normal room temperature for further studies.

Cow dung cake

The neem leaves were collected from Morigaon district in the month of April 2024 (26.2600° N, 92.2630° E), and the cow dung was collected from the cow shields of different areas of Morigaon district. The prepared neem extract was then taken, mixed with cow dung, and plated accordingly. The wet-weights of the cakes were recorded. For the further determination of dry weight, it was allowed to dry in the sun for 24 hours, and weight was taken.

Cage test for natural mosquito repellent spray

The cage test is the most common way of testing the effectiveness of a mosquito repellent spray. To evaluate the effectiveness of mosquito repellent spray activity against mosquitoes, we performed preparatory experiments with mosquitoes in cages. The mosquitoes used in this experiment were caught using a net while biting humans between 6 p.m. and 9 p.m. 25 mosquitoes were placed in the cage (B. Prabasheela *et. al.*). Prior to testing, volunteers washed their hands with unscented soap. Treatment comprises a repellent spray applied to the forearm of a volunteer skin surface area. The treated forearm is inserted into the cage and exposed to mosquitoes. The number of mosquitoes that land and probe the skin in 15 minutes was observed and recorded. The observations are repeated every hour.

Evaluation

During the investigation of mosquito repellent activity, the prepared repellents were checked for their flammability, burning efficiency, application with respect to burning time, and eventually their effective repellent activity. The application of these natural repellents was conducted to check their consistent combustibility. Further, the time taken to burn the cow dung cake, the smoke produced, and its causal effects such as irritation, coughing, and tears were observed and recorded. Ash produced by cow dung was weighed and recorded. The cow dung was burned in the evening and night, such as shrubs, cow shield, etc. Mosquito repellent spray was used on the skin to check its consistency.

Larvicidal bioassays

Neem and marigold extract were used as a treatment group, while a control group was also taken. The number of dead larvae is recorded after a specified time period (e.g., 24 hours) of exposure to the treatment.

Irritancy Test of spray

The prepared spray was then tested as per standard protocol.

Irritancy, erythema, and edema were checked for, if any, at regular intervals up to 24 hours and reported.

Determination of pH

The pH metre was calibrated using a standard buffer solution. The pH of the spray was measured at room temperature using the pH metre.

Phytochemical screening of the methanolic extract of *Azadirachta indica* and *Tagetes erecta*

The crude extracts of leaves were subjected to preliminary phytochemical screening for possible presence of bioactive compounds. The following tests were performed on the extracts to detect various phytoconstituents present in them.

Test for tannins

Potassium dichromate test: To a 1 ml of extract in a test tube and few drops of 5% ferric chloride was added and observed for the brown colour or dark green colour.

Test for steroid

Salkowski reaction: 1 ml of extract was shaken with chloroform in a test tube; few drops of acetic anhydride were added and boiled in a water bath and rapidly cooled in iced water. Conc. Sulphuric acid (2mL) was added alongside of the test tube. Formation of yellow colour and turning the upper layer to green showed the presence of steroids.

Test for Terpenoid

Liebermann-Burchard test: To a 1 ml of extract, 2 ml chloroform of was added. 3 ml conc. Sulphuric acid was carefully added to the layer, and observed the brown colour.

Test for saponins

Froth test: The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of froth showed the presence of saponins.

Test for alkaloids

Wagner's test: 1 ml of extract was taken in test tubes and 2-3 drops of Wagner's reagent was added. Reddish brown colour precipitation indicates the presence of alkaloids.

Test for Flavonoid

Alkaline reagent test: To 1 ml of the extract, a few drops of dilute sodium hydroxide were added. An intense yellow colour was produced, which become colourless on addition of a few drops of dilute acid indicates the presence of Flavonoid.

Phenolic compounds

Ferric chloride test: The extract was dissolved in alcohol and 1 drop of neutral ferric chloride was added. The intense black colour indicates the presence of phenolic compound.

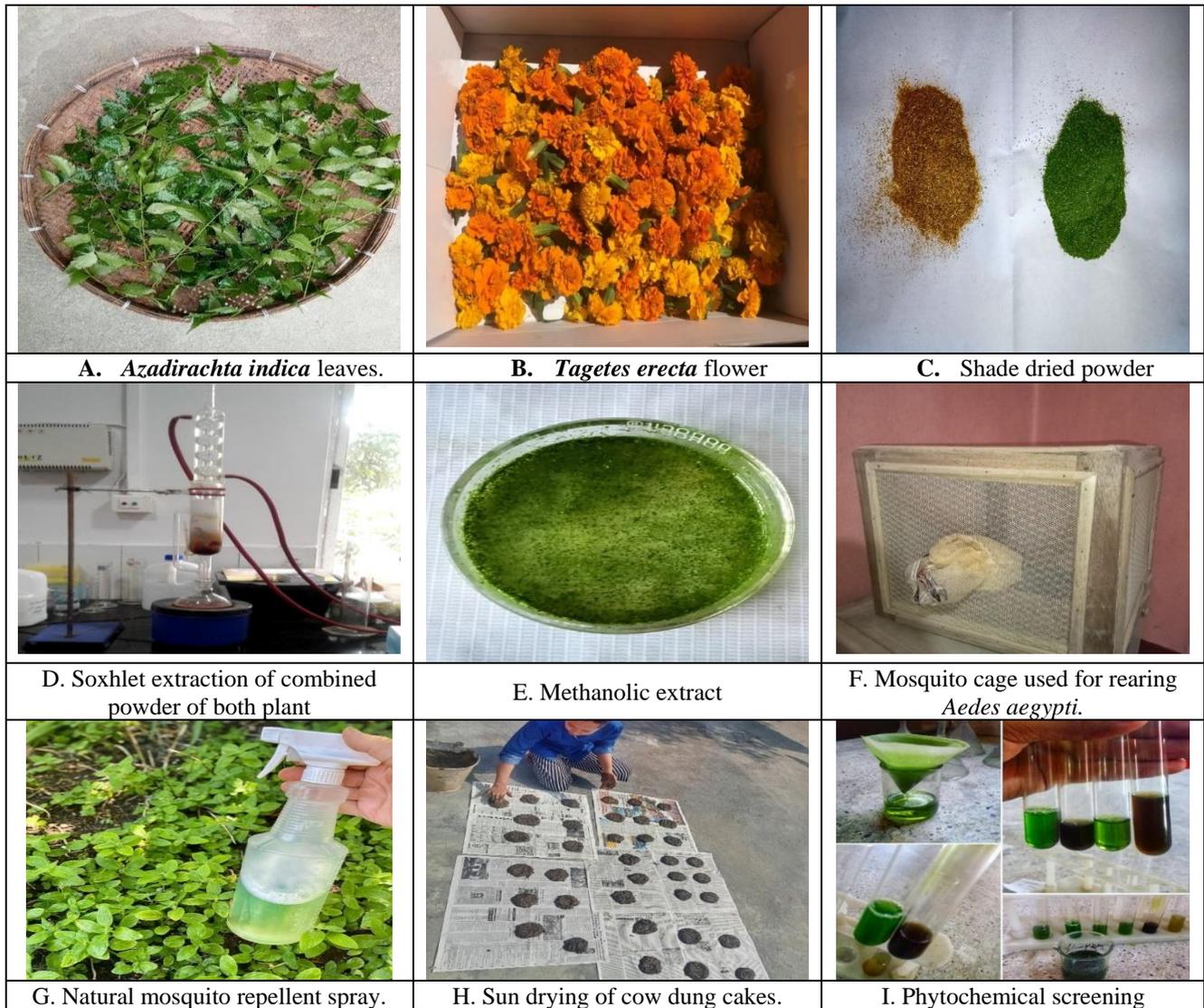


Fig 1: Figure showing plant extract preparation(A-E), Mosquito rearing(F), Repellent preparation (G, H) and Phytochemical screening of plant extract(I).

Results and discussion

In the present study, we aimed to formulate a natural, herbal mosquito repellent based on neem and marigold flower, which are natural sources of essential oils. Among the plant families with promising essential oils used as mosquito repellents, individual compounds present in these mixtures with high repellent activity include limonene, camphor, thymol etc. These herbal products have the potential to provide effective and safer repellents for humans and the environment.

In this present investigation the colour, odour, skin irritability and pH were determined and listed in Table:1.

Table 1: Primary observation of herbal mosquito repellent spray

Parameters	Formulations
Colour	Green
Odour	Mild
Skin irritation	No irritant
pH	7.6

Table 2: Evaluation of Cage test

Time (Hours)	Number of Mosquitoes Landing and Probing (Control)	Number of Mosquitoes Landing and Probing (Treated)	Percentage Repellency
1	20	5	75%
2	22	7	68.2%
3	25	10	60%
4	20	12	40%

Evaluation of cow dung cake

An attempt has been made here to prepare cow dung cake using cow dung and neem leaf paste. Table 3 shows the primary observations of the prepared herbal formulation. The volume of the smoke exhibited by the mosquito

repellent cow dung cake shows promising results, which explains the efficacy of natural insecticidal preparations than synthetic repellents. The same formulations were also tested for mosquito repellency in mosquito-affected cow shields. During the burning of herbal mosquito cow dung

cake, it was shown that up to 90% of the mosquito population was greatly reduced. The formulations contain neem, which emerged as the best composition with a good aroma.

Table 3: Primary observation of cow dung cake

Burning time (minutes)	Ash weight (gm)	Irritability	Odour	Smoke visibility
40	1.54	No	Smoky	Average

Table 4: Mosquito population reduction by cow dung cake with neem leaf paste

Time (minutes)	Mosquito population reduction (%)
15	80
30	85
60	90
120	80

The cow dung cake analysis reveals a uniform, natural, and organic product with potential uses as fuel or fertilizer. The consistent of efficient burning, minimal pollution indicate a high-quality product.

Phytochemical analysis

The methanol extract was subjected to phytochemical screening using various chemical tests to detect the presence of different bioactive compounds. The result of preliminary phytochemical analysis showed the presence of phytochemical like tannin, steroid, terpenoid, flavonoid, phenol, saponin, and alkaloid.

Table 5: Phytochemical screening

Test	<i>Azadirachta indica</i>	Marigold
Tannin	+	+
Steroid	+	+
Terpenoid	+	+
Alkaloid	+	+
Flavonoid	+	+
Phenol	+	+
Saponin	+	+

The present study revealed that methanol, leaves extract contained Flavonoid, phenols, Saponin, steroids, tannin, Terpenoid, and Alkaloid in solvent.

Statistical analysis

The phytochemical screening results were analysed using descriptive statistics. The presence or absence of each phytochemical constituent was recorded and presented in a tabular form.

Percentage phytochemical constituents of methanol extract of *Azadirachta indica* and *Tagetes erecta*

Phytochemical	Yield (%)
Tannin	2.63±0.07
Steroid	0.68±0.10
Terpenoid	2.57±0.05
Alkaloid	3.21±0.07
Flavonoid	1.94±0.06
Phenol	1.48±0.02
Saponin	1.20±0.04

The table presents the percentage composition of various phytochemicals present in the methanol extracts of *Azadirachta indica* and marigold flower. These phytochemicals are known for their medicinal properties, including antioxidant, antimicrobial activities.

Discussion

As per Makhaik *et al.*, (2005), neem plants have been traditionally used to repel mosquitoes in jungle regions. Palanisami *et al.*, (2014) observed that neem with cow dung showed good mosquito repellent activity in performed tests as well as a strong mosquitocidal agent. Tripathi *et al.*, (2009) found that the plant has been widely recognised for its ethnobotanical and medicinal usefulness. Neem and cow dung in general have been recognised as an important natural source of insecticides, larvicides, and repellents. Therefore, the use of neem with cow dung for the effective control method for minimising the noxious effects of some pesticide compounds on the environment.

Conclusion

Investigation of natural mosquito repellents and their possible effects have attracted the attention of many researchers for ages. This study eventually led to the plant-based mosquito repellent discovery process. The outcome expected from this research will hopefully contribute to the development and utilisation of the natural resources of Assam and other parts of northeast. It will also contribute to rural development through proper utilisation and bioactivities. This formulation allowed for new, more efficient forms of natural mosquito repellents.

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