Indian Journal of Fibre & Textile Research
Vol. 48, September 2023, pp. 346-352
DOI: 10.56042/ijftr.v48i3.6060

Sustainable mosquito repellent finish on cotton using *Eucalyptus globulus* leaf dye extracts

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Received 6 September 2022; revised received and accepted 25 October 2022

An attempt has been made to develop textiles, which can actively repel the mosquitoes from entering into the rooms by imparting nanoparticles in the form of prints. The active material used in the study is eucalyptus leaf nanoparticles in two forms, viz pre and post calcinated, each in two concentrations. The samples printed with nanoparticles are compared with the control sample (printed without nanoparticles) for mosquito repellent efficacy against Anopheles mosquitoes, using the modified WHOPES Excito chamber test method. The developed samples are also tested for durability to laundry until 15 wash cycles. The results show that the printed samples using eucalyptus leaf extracts in combination with nanoparticles in two forms have significantly higher mosquito-repellent efficacy. The study also reveals that 90% efficacy is achieved with 10% pre and post calcinated nanoparticles. The finding implies that samples printed with 10% pre and post calcinate nanoparticles have high mosquito-repellent efficacy even after 10 wash cycles and have commercial viability. These fabrics can be used for curtains and screens for doorways to reduce the entry of mosquitoes.

Keywords: Cotton, Eco-friendly prints, *Eucalyptus globulus*, Functional prints, Mosquito repellent fabric, Nanoparticles, Textile finishes

1 Introduction

Functional or special finishes are designed to protect the wearer from wind, water, stains, abrasion, dust, microorganisms, insects, UV rays as well as other radioactive radiations, static electricity, etc.[1]. Finishing the textile material using natural sources is gaining popularity these days and various properties like mosquito repellent finish, anti-microbial finish, anti-septic, water repellent, self-cleaning and wound healing properties can be incorporated into the textile materials by finishing it with inherentsources[2]. Use of natural sources in the development of mosquito repellent finishes to textiles is highly required as mosquitoes are dangerous insects that transmit deadly diseases like malaria, dengue, filariasis, zika, etc. to humanity and additionally, the chemicals used in synthetic mosquito repellents are detrimental to the public as well as the environment[3,4]. Various techniques to inoculate mosquito-repellent agents to textiles include polymer coating, microencapsulation, absorption and incorporation[5]. Many studies reported the technique of pad dry cure for treating microencapsulated repellents either from natural or chemical sources onto fabric[6-8].

Due to their exclusive physical and chemical characteristics, in the past few years, nanotechnology has gained the highest notch in technical, pharmaceutical, ecological and medical applications[9, 10]. Currently, for production of nanoparticles, green synthesis method is given more prominence, because it is considered as a thrifty, environmentally safe method that consumes less time and provides homogenous composition of particles with high yield[11, 12].

Further, the growing consciousness of people towards natural dyed and printed textiles and products is gaining market place[13-15]. The present study focuses on developing environment-friendly mosquito-repellent printed textiles crafted with nanotechnology using eucalyptus leaf extracts. Furthermore, the durability of mosquito repellent finish to laundering is also analysed.

2 Materials and Methods

The experimental procedure was conducted under standard atmospheric conditions (20°C, 65% RH) at All India Coordinated Research Project Laboratory,

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Professor Jayashankar Telangana State Agricultural University, Hyderabad.

2.1 Synthesis of Nanoparticles

Nanoparticles were synthesized using fresh eucalyptus leaves through green synthesis method at Nano Science and Technology Laboratory, Jawaharlal Nehru Technological University, Hyderabad.

The first step in the synthesis of nanoparticles is extraction, the aqueous method was employed using fresh leaves of eucalyptus. The leaves were rinsed thoroughly and boiled using distilled water at 60° C for 1-2 h with material-to-liquor ratio of 1:5, which was then filtered using Whatman no. 41 filter paper[16].

The precursor used for the synthesis of nanoparticles is titanium dioxide (TiO_2)[17,18]; one molarity of TiO_2 was dissolved in 100 mL distilled water which was stirred continuously on a magnetic stirrer at 400 rpm (rotations per minute). After one hour of constant stirring, 40 mL of plant extract was added slowly to this and the whole solution was allowed to stir for 4 h. This whole solution is allowed to settle overnight and centrifugated to remove the by-products. The wet cake/ slurry which was deposited after processing was dried up in a hot air oven at 70° C for 12 h to obtain nanoparticles in powder form which is referred to as pre-calcinated nanoparticles[19]. The obtained powder was further calcinated at 200° C for 2h in a muffle furnace, to later acquire calcinated nanoparticles [20].

2.2 Characterization of Nanoparticles

Bruker D8 X-ray diffractometer was used for the analysis of the crystalline structure of the nanoparticles. The average crystalline size was calculated using the following Debye- Scherrer's formulae:

$$D = \frac{K\lambda}{\beta . Cos\theta}$$

where D represents the average grain size of the material; K, the Debye Scherrer's constant (=0.94); λ, the wavelength of the radiation; β, the full width half maximum of the peak; and θ, the Bragg's angle.

HORIBA SZ-100 particle size analyser was used to measure the average size of the particle. The FTIR spectral analysis was carried out to identify the functional groups present in nanoparticles. Particles were scanned under scanning electron microscope (SEM – Model: ZEISS EVO MA 50) at a required magnification of ×500 – k×50.00, as per the standard procedures in order to calculate the size of the nanoparticles[21, 22].

2.3 Material Used for Printing

Hundred per cent cotton fabric in plain weave with 122 GSM and 200 thread count was used for the study.

2.3.1 Desizing, Scouring and Pre treatment

De-sizing and scouring of the fabric were carried out to take off the impurities that might inhibit the absorption of dye and nanoparticles. For effective fixation of natural dye compounds, desized fabric was pre-treated using myrobalan powder solution for dye fixation[23]. The solution was prepared by soaking 20 g of myrobalan powder (for 100 g of the fabric) for 4 h with a material-to-liquor ratio of 1:30. The desized fabric was soaked in the solution overnight, squeezed and dried in sunlight[24, 25].

2.3.2 Extraction of Dye

In the current study, standardised extraction and printing procedures with natural dyes for cotton were adopted based on the research findings of Scientists of the Indian Council of Agricultural Research, All India Coordinated Research Project, Hyderabad[26].

Fresh eucalyptus leaves were weighed, rinsed, steeped overnight and boiled in water with the material-to-liquor ratio of 1:10 at 80°C for 45 min. The obtained extract (dye) was strained and reduced to 40% (w/w) aqueous extract, which was used for printing further[27,28].

2.3.3 Gum Extraction and Selection of Mordant

Gum essential for printing was prepared in the required consistency from dried seed powder of *Cassia tora* with a material: liquor ratio of 1:20. Mordanting is the treatment given to the textile fabrics with different metallic salts which helps in binding the natural dye onto the textile fibres[29,30]. This was usually performed in three methods pre-mordanting, post mordanting and simultaneous mordanting, out of which simultaneous mordanting was selected for the study. Different types of mordants deliver distinct colours for the same dye[31,32]. Copper sulphate was selected as a mordanting agent for the present study.

2.3.4 Preparation of Print Paste

The print paste was prepared by adding dye to the gum in 4:3 ratio to which copper sulphate (mordant) was added at a concentration of 0.8g/100 g. Five different types of mosquito repellent print pastes were formulated with the addition of nanoparticles in 5% and 10% in two different forms (pre and post calcinated) separately to the print paste.

The mosquito repellent paste was diffused onto the pre-treated cotton fabric by screen printing method.

2.3.5 Post Treatment

The printed fabrics were post treated to improve the fastness properties. The process of post treatment included shade drying for 2 days, steaming at 100° C using autoclave and steeping in 5 % sodium chloride solution trailed by rinsing with 2 gpL neutral detergent solution and lukewarm water followed by shade drying. This process enabled the nanoparticles to adhere well to the fibre, strip off the excess nanoparticles and dye from the fabric surface and enhances the fastness properties[33-36]. The fabrics printed were tested for mosquito repellence both before and after post treatment, in order to check the percentage loss of efficacy.

2.3.6 Conditioning and Laboratory Testing of Samples

All the printed fabrics were subjected to various laboratory tests to assess their functional performance. The fabric samples before and after post treatment were conditioned for 24 h in a standard atmosphere of 65 ± 2% relative humidity and of 21° C ± 2° C following ASTM 1776 and Bureau of Indian Standards IS 6359 – 1977 before testing[37]. In the present study, WHOPES Excito chamber test method was used for testing the mosquito repellent property. This is a modified conventional method of observing the change in the behaviour of mosquitoes when exposed to treated fabrics, in the form of moving away from the treated to untreated fabric holding chamber. Modified WHOPES Excito chamber test method consisted of two connected acrylic chambers, each chamber measuring 30cm × 30cm × 30cm with a middle exit portal which can be opened and closed when needed. After allowing 30 mosquitoes in the chamber holding treated fabric, observations were documented after 30 min exposure and repeated four times for each sample. The observations like the number of specimens that escaped to the chamber holding untreated fabric and those remaining in the chamber holding treated fabrics were noted separately for each exposed chamber. The mosquitoes escaped to the untreated fabric chamber and those remaining in the chamber of treated fabric were held discretely in breathable containers provided with food and water to mark the mosquito mortality[38-40].

Efficiency of the mosquito repellent percentage for each sample was calculated using the following formulae:

$$\frac{\text{No. of specimen escaped} + \text{No of specimen dead}}{\text{No. of specimen exposed}} \times 100$$

Following codes were given for the samples to easily recognize and avoid repetition of sentences:

- C (control) – fabric printed with dye from eucalyptus leaf extract (without adding nanoparticles),
- BC5–fabric printed with dye from eucalyptus leaf extract added with 5% pre-calcinated eucalyptus nanoparticles,
- BC10–fabric printed with dye from eucalyptus leaf extract added with 10% pre-calcinated eucalyptus nanoparticles,
- AC5–fabric printed with dye from eucalyptus leaf extract added with 5% post calcinated eucalyptus nanoparticles,
- AC10–fabric printed with dye from eucalyptus leaf extract added with 10% post calcinated eucalyptus nanoparticles,

The retention of mosquito repellent efficacy until 15 washes for all the samples was tested using standard procedures. The mosquito-repellent percentage of washed samples after each wash was compared with the results of unwashed fabric samples.

2.4 Statistical Analysis

The modified WHOPES Excito chamber test method was used with four replications for each sample and the number of mosquitos present in the chamber was noted after 30 min exposure, to calculate mosquito repellency percentage. One-way analysis of variance at α=0.05 was used to test the equality of mean mosquito repellency percentages among samples for both before and after post treatment. For knowing the effect of wash cycles on repellence percentage, a two-way analysis (α=0.05) was carried out taking different samples as treatment and wash cycles as blocks.

3 Results and Discussion

The results of XRD reveal that the average grain size formed in the biosynthesis of calcinated nanoparticles has been determined using Debye Scherrer's formulae as 15.2 nm. The average particle size is noted as 42 nm for pre-calcinated and 30 nm for post calcinated (200°C) TiO_2 nanoparticles when tested using a particle size analyzer. The FTIR (Fourier transform infrared spectroscopy) spectra of the eucalyptus (TiO_2) nanoparticles show O-H stretch and aromatic ring vibrations in both the forms of nanoparticles. The presence of C-O stretch (1108 cm^{-1}) of terpenoid components is identified in pre-calcinated nanoparticles. Other compounds like aliphatic fluoro carbons, alcohols, and phenols are revealed at different wavelengths. The results of the FTIR analysis are strengthened with the results of Elzey *et al.* [41], who reported the presence of alkaloids, phenols and terpene compounds (terpineol, cineole and citronellal) in eucalyptus leaf extracts which are found responsible for antibacterial and mosquito repellent activity.

The morphological study of synthesized nanoparticles is done using a three-dimensional image produced by scanning electron microscope (ZEISS EVO MA-50) from × 500 - k× 50.00 magnifications with an accelerating voltage of 10 kV. The results of the SEM analysis reveal the particle size as 120-172 nm for pre-calcinated and 66-100 nm for calcinated eucalyptus nanoparticles respectively.

The samples when printed using two forms of nanoparticles having two different percentages display differences in tints and shades. The colours obtained are coffee brown for the control sample, cinnamon brown for BC5, walnut brown for BC10, cedar brown for AC5 and umber brown for AC10. These samples are tested for mosquito repellent efficacy using a modified WHOPES Excito chamber test method and the results are represented in Fig. 1 and Table 1.

Table 1 — Mosquito repellent efficacy of fabrics printed with *Eucalyptus globulus* (leaves) nanoparticles

Samples	Before post treatment	After post treatment
BC-10	89.75^{a}	89.75^{a}
AC-10	89.75^{a}	86.25^{ab}
AC-5	86.25^{a}	83^{b}
BC-5	86.25^{a}	86.25^{ab}
C	76.25^{b}	73^{c}
	F-value=14.87***	F-value=16.21***
	CD=4.33	CD=4.80

Samples sharing the same superscript letter (e.g. a, a) signify that their means are statistically at par, indicating no significant differences between them. Conversely, sample means distinguished by different superscript letters (e.g. a, b, ab, c) indicate significant difference between those respective samples. *** indicates the significance at 0.1% level of significance.

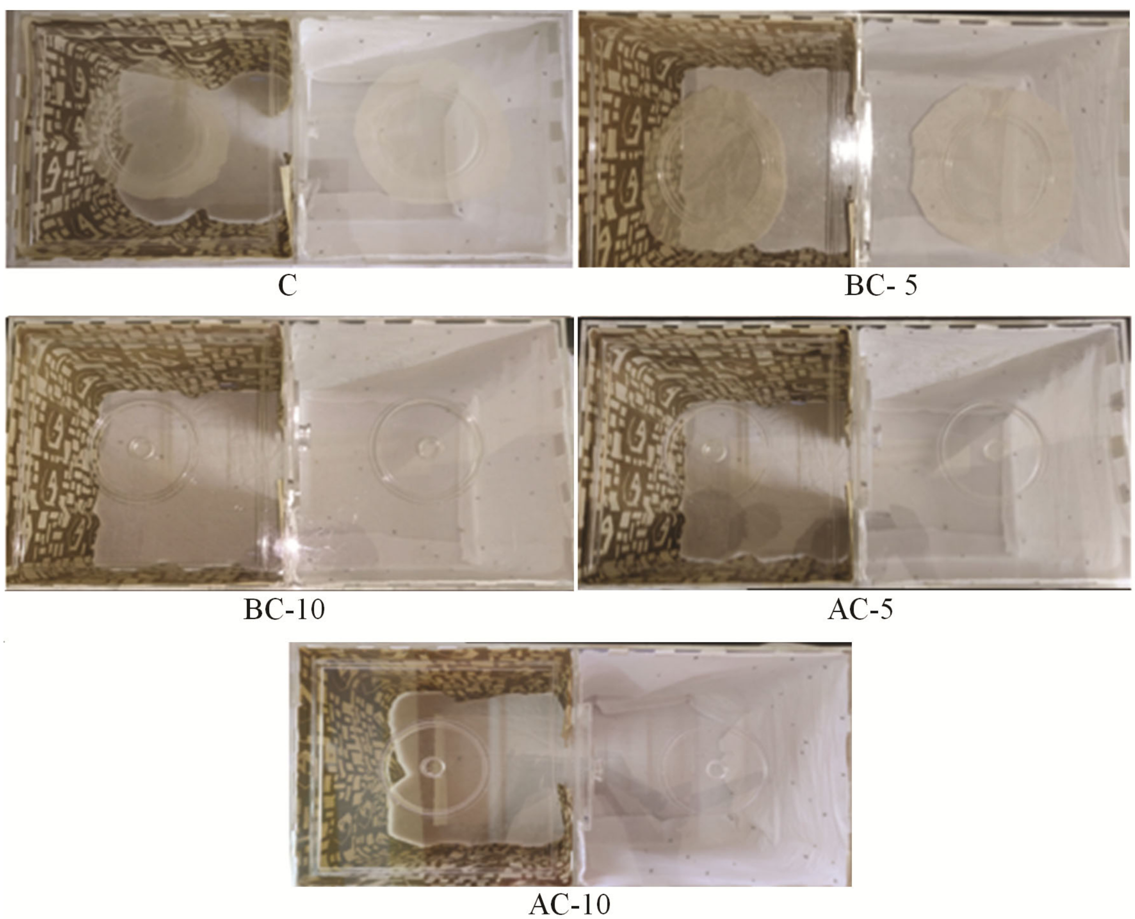

Fig. 1 — Mosquito repellent activity of samples using Excito chamber test method

The results of the Excito chamber mosquito repellent test, presented in Table 1, reveal that the mosquito repellent efficacy of fabrics printed with *Eucalyptus globulus* (leaves) nanoparticles is significantly higher than the control sample both before and after post treatments at less than 1% level of significance. Further, the findings in Table 1 indicate that, samples before post treatment record 89.75% mosquito repellent efficacy followed by 10% pre and post calcinated nanoparticle printed samples. The samples printed with 5% nanoparticles in both pre and post calcinated forms showcase 86.25% efficacy. These samples are not significantly different from each other but are significantly different from the control sample at less than 1% level of significance which is also represented in Fig. 2. The difference between the samples printed with and without nanoparticles may be due to the presence of nanoparticles that modifies the surface area, thereby providing active area for repelling mosquitoes.

When the repellence percentage is tested and noted after post treatment, the samples printed with 10% before calcinated nanoparticles show an efficacy of 89.75 per cent. Samples printed with both pre and post calcinated nanoparticles with 5 and 10% respectively display a repellency percentage of 86.25%. Whereas the samples printed with 5% post calcinated nanoparticles displayed 83% efficacy and the control sample displayed 73% mosquito-repellent efficacy. One-way analysis of variance presented in Table 1 reveals that all the samples printed with nanoparticles are significantly different from the control at less than 1% level of significance and the sample printed with 5% post calcinated nanoparticles is significantly different from pre calcinated sample printed with 10 % nanoparticles (Fig. 3).

All the samples have showcased a reduction in mosquito repellent efficacy after post treatment, except the samples printed using pre-calcinated nanoparticles. Further, the reduction in mosquito repellent efficacy by 3.5% is noted in sample printed with 10% post calcinated nanoparticles and also dropped by 3.25% in two samples, viz 5% post calcinated sample and control sample respectively.

The mosquito repellent printed samples are assessed for wash durability of prints and tested for mosquito repellent efficacy using Excito chamber test over 15 wash cycles. The results of two-way analysis (Table 2) reveal that the samples printed with nanoparticles have significantly high mosquito repellency than the control sample at less than 1% level of significance. The samples printed with 10% pre calcinated nanoparticles maintained the highest repellency with an average of 61% during 15 wash cycles followed by 10% post calcinated sample, 5% pre calcinated sample, 5% post calcinated and control samples with 58.4%, 57.4%, 51.8% and 27.6%

Table 2 — Mosquito repellent efficacy of fabrics printed with Eucalyptus globulus (leaves) nanoparticles after wash cycles

Samples	Means	Wash cycles	Means
BC-10	61^{a}	1	76.6^{a}
AC-10	58.4^{a}	3	63.6^{b}
BC-5	57.4^{ab}	5	53.6^{c}
AC-5	51.8^{b}	10	40.6^{d}
C	27.6^{c}	15	21.8^{e}
	F-value=49.67***		F-value=118.98**
	CD=5.80		CD=5.80

Samples sharing the same superscript letter (e.g. a, a) signify tha their means are statistically at par, indicating no significar differences between them. Conversely, sample means distinguishe by different superscript letters (e.g. a, b, ab, c, d, e) indicat significant difference between those respective samples.
*** indicate the significance at 0.1% level of significance.
The CD value is the same for samples and wash cycles as th MSE (mean square error) was obtained using two-way ANOV with five samples as treatments and five wash cycles as blocks.

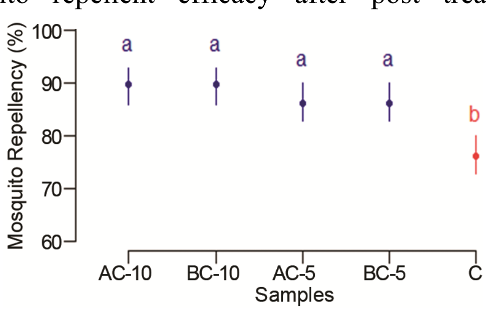

Fig. 2 — Mosquito repellent percentages of samples before post treatment with *Eucalyptus globulus* (leaves) nanoparticles [samples with same letter are statistically equivalent, and sample with distinct letters have significant differences]

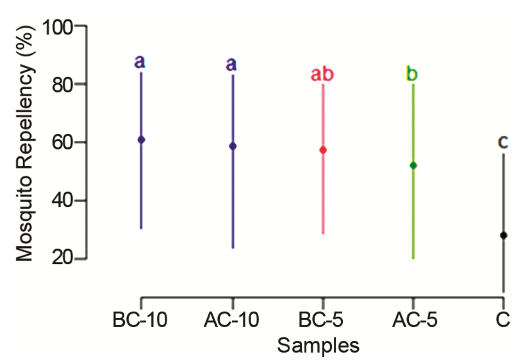

Fig. 3 — Mosquito repellent percentages of post treated samples with *Eucalyptus globulus* (leaves) nanoparticles [samples with same letter are statistically equivalent, and sample with distinct letters have significant differences]

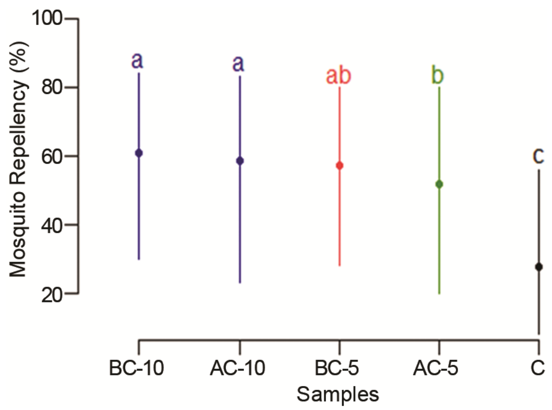

Fig.4 — Mosquito repellent percentages of samples printed with *Eucalyptus globulus* (leaves) nanoparticles during wash cycles [samples with same letter are statistically equivalent, and sample with distinct letters have significant differences]

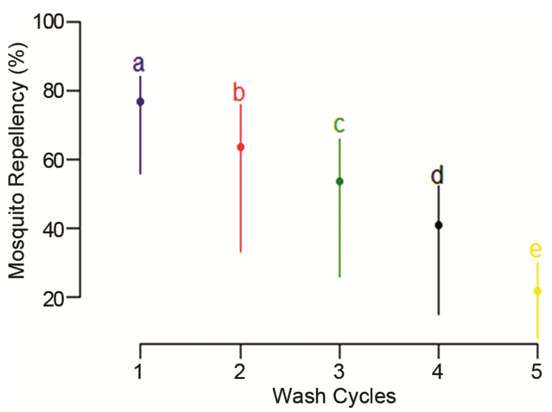

Fig. 5 — Mosquito repellency percentages among different wash cycles in samples printed with *Eucalyptus globulus* (leaves) nanoparticles [samples with same letter are statistically equivalent, and sample with distinct letters have significant differences]

respectively. Figure 4 also confirms the same with the control maintaining significantly lower repellency than other samples. The results in Table 2 also indicate that there is a highly significant decrease in mosquito repellent percentages after each wash cycle with samples maintaining more than 50% average mosquito repellency only till 5th wash cycle. Figure 5 confirms the significant difference in mosquito repellency among the wash cycles. The results correspond to the findings of Maheshwari and Ramya[42], who reported a reduction in repellency after repeated washes from 96 % to 40 % with direct application method and from 94% to 52% with microencapsulation method in samples finished with *Andrographis paniculata* plant extracts.

Overall, the study establishes the fact that fabrics printed with only dye extract (control) experience extreme reduction in repellence after each wash during 15 wash cycles. Whereas, fabrics printed with 10 % pre-calcinated nanoparticles record the best results which are durable till 15 wash cycles followed by samples printed using 10% post calcinated and 5% pre-calcinated nanoparticles. The results clearly indicate that wash durability of treated samples is directly proportional to percentage of nanoparticles used. The gradual reduction in repellence percentage with increase in wash cycles may be attributed to the loss of active compounds due to pressure and detergent applied during washing. The results exhibited in the study corroborate with the results of Apoorva and Archana[43], who developed natural mosquito-repellent cotton fabric using sweet lime peel extracts. With an increase in concentration of extract and padding time, mosquito repellent percentage is enhanced and retained till 6 wash cycles.

4 Conclusion

The present work focused to develop mosquito repellent printed textiles by applying nanoparticles extracted from eucalyptus leaves. The finish is applied to the textiles in the form of prints, so as to provide decorative appeal added to the functional performance of the fabrics. The study proves that, the nanoparticles have an active role in repelling mosquitoes and they directly contribute to the durability of the finish. The implications of the study are, mosquito repellent finishes can be developed with the addition of nanoparticles to the print paste and can be value added on the fabrics aesthetically in the form of prints, so as to reduce man-vector contact. The findings of the study also have commercial viability in the textile industry. Textile material with mosquito-repellent prints can be used for draperies and furnishings for homes, hotels, resorts, etc.

Acknowledgement

The authors are thankful to the Head, Department of Nano Science and Technology, Jawaharlal Nehru Technological University, Hyderabad for providing facilities and permission to prepare the nanoparticles.

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