ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 09, Issue, 10, pp.8782-8785, October, 2018

RESEARCH ARTICLE

MICROENCAPSULATION OF HERBAL-MICROBIAL PIGMENTS: A STUDY ON ANTIBACTERIAL AND LARVICIDAL PROPERTIES OF THE DEVELOPED COMPOSITES

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ARTICLE INFO

Article History:
Received 15th July, 2018
Received in revised form
20th August, 2018
Accepted 12th September, 2018
Published online 30th October, 2018

Key words:
Microencapsulation, Gliciridiasepium, Pseudomonas fluorescenceas.

ABSTRACT

The present research work was carried out by finishing herbal extracts of *Gliciridiasepium* and bacterial pigment extracts of *Pseudomonas fluorescence*as a novel composite mixtures. Finishing was done on two different materials, tencel and viscose. The aim of this research is to impart a mosquito repellent and antibacterial finish and to evaluate its functional properties like mosquito repellence, and antibacterial activity. Following objectives were framed in this present research to fulfil the aim of the study.

Citation: ***Varsha*, 2018.** "Microencapsulation of herbal-microbial pigments: A study on antibacterial and larvicidal properties of the developed composites", *Asian Journal of Science and Technology*, 09, (10), 8782-8785.

INTRODUCTION

Health and hygiene are the primary and foremost equirements for human beings to live comfortably and work with maximum efficiency. To protect mankind from pathogens and to avoid cross infection, a special finish like antimicrobial finish has become necessary. As consumers have become more aware of hygiene and potentially harmful effects of micro organisms, the demand for antimicrobial finished clothing is increasing. In selecting the active substances for hygienic finishing, it must be ensured that these substances are not only permanently effective, but also compatible with the skin. Microbial infestation is a serious cause with clothing worn especially next to the skin. Antibacterial fibers and various antibacterial chemicals available in international market are mostly from synthetic base and are not environment friendly. Consumer preference has changed and higher demands are placed on the functional fabrics that makes them feel comfortable even when they wear for a whole day. These new requirements necessitate a production process that is environmental friendly. There are many natural plant products, which show antibacterial properties. Extract from roots, stem, leaves, flowers, fruits and seeds of diverse species of plants exhibit antibacterial properties. These antibacterial extracts can be used as textile finishing agents in the crude form or as microcapsules to enhance the durability and controlled release of the extracts.

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Microencapsulation is a rapidly expanding technology and finds greater applicability in textiles in recent years. Uniqueness of microencapsulation is the smallness of coated particles and it provides a means of packing, separating and storing materials on a microscopic scale for later release under controlled conditions. In the present work, herbal plants extracts of Gliciridia sepium and pigment extract of Pseudomonas fluorescence are were blended together to form a novel composite mixtures applied onto twill fabric as antimicrobial finishing agent. Microcapsules are produced using herbal extract as core and acacia as wall material. Comparison between directly applied and microcapsualted herbal extract is done in terms of antimicrobial efficacy and durability.

MATERIALS AND METHODS

100% Tencel and 100% Viscose fabric (3 mtr each) with twill weave structure was used for antimicrobial and larvicidal properties. Herbal plants extracts of Gliciridia sepium and pigment extract of Pseudomonas fluorescence were blended together to form a novel composite mixtures applied onto twill fabric as antimicrobial finishing agent. These plants were collected in and around Palakkad, Kerala India. Fresh leaves of the collected plants were washed thoroughly under running tap water and in sterile distilled water. The leaves were cut into small pieces and air dried for 12 - 15 days in the shade for minimum of 1 week. The shade dried leaves were pounded to coarse powder in a low speed blender and stored in air tight

container at room temperature till future use. 1 ½ meter for lab work/testing and 1 ½ meter for product development , two types of fabric were used in the present research such as 100% Tencel fabric (Twill weave structure) and 100% Viscose fabric (Twill weave structure). The macroscopic and stereo zoom microscopic images of the selected fabrics are presented below as separate images. Microbial pigment was synthesized, extracted from the selective bacteriological media. Test bacterial culture, Pseudomonas fluorescence was used in the study based on their high pigment producing abilities naturally. The bacteria was inoculated in the Nutrient broth (culture media) and incubated for 48h to 72h till high pigment production. After observing the change in colour of the media, the entire broth culture was centrifuged at 5000 to 8000rpm for 30 min repeatedly for two times. The supernatant containing the fluorescent pigment was collected separately in a glass container and stored at 4°C prior use. About 500g of each Gliciridia sepium powdered leaves were extracted with 1.5L of acetone separately using Soxhlet extraction apparatus for 2 days with regular intermittent intervals. The extract was evaporated to dryness using evaporator. Herbal extract was stored in the refrigerator prior to use.

Herbal extracts of Gliciridia sepium and pigment extracts of Pseudomonas fluorescence were blended together to form a novel composite mixtures. For 1:1 herbal and bacterial pigment composite preparation, 500ml of herbal extract was dispersed in 500ml of bacterial pigment solution. Herbal extracts was added into the bacterial pigment using a standard burette measuring 100ml. The flow rate of herbal extract from the burette was kept constant in such a way to deliver 10ml of extract into the bacteria pigment solution. During the addition of herbal extract, the bacterial pigment was kept at stirring condition (120 rpm) using a magnetic stirrer. The developed composite mixture was stored at 4C prior to use. Further the composite was used for antibacterial assay, antilarvicidal assay, and mosquito repellent assay. Selected Fabric – 3 meter each Developed herbal : bacterial pigment composite (core materials) Material liquor ratio - 3:3 Sodium alginate (wall material) - 3% Time - 2 hours Temperature - 40 °C Solvent - Calcium chloride Microcapsules containing composite pigment extract as core material and sodium alginate as the wall material was prepared as follows. About 100g of wall material was allowed to swell for half an hour by mixing with 100 ml of hot water. To this mixture 50 ml of hot water was added and stirred for 15 minutes maintaining the temperature between 40°C and 45°C. To this mixture, 100 ml of core material (composite) was added at a stirring condition (100-100 rpm) for 15 minutes. This was sprayed into 2% of calcium chloride solution using a spray gun (0.5mm pore size) fixed with in an air-compressor (0.5horse power). The droplets were retained in calcium chloride collection bath for 15 minutes. In this bath the calcium ions diffused with the alginate solution, thereby hardening the matrix and forming a solid hydro gel system. The microcapsules were obtained by decantation and then used for finishing on the selected fabrics by exhaust method.

Qualitative tests - Parallel streak method Test specimens (composite finished fabric) were cut into pieces (25mm x 50mm). A 50mm length permits the specimens to lay across 5 parallel inoculums streaks each of diminishing width from both 8mm to 4mm wide. Sterile AATCC bacteriostasis agar plates were prepared. Using sterile 4mm inoculating loop, one loop full of culture (Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 6538) was loaded and transferred to the surface of the agar plate by making five parallel inoculum streaks spaced 10mm covering the central area of the Petridis without refilling the loop. The test specimen was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with agar surface. The plates were incubated at 38°C for 16-24 hours. The inoculated plates were examined for the interruption of growth along the streaks of inoculums beneath the fabric and for a clear zone of inhibition beyond the fabric edge. The average width of the zone of inhibition around the test specimen calculated in mm. Among the three fabric samples, one best concentration was selected for quantitative antibacterial assay (AATCC Test method 100-2012). This test method provides a quantitative procedure for the evaluation of the degree of antibacterial activity. Assessment of antibacterial finishes on textile materials is determined by the degree of antibacterial activity intended in the use of such materials on the fabric. Test and control swatches are inoculated with the test organisms at a standard temperature. After incubation, the bacteria are eluted from the swatches by shaking in a known amount of neutralizing solution. The number of bacteria present in this liquid is determined, and the percentage reduction by the treated specimen is calculated.

The assay used for measuring antibacterial properties was based on the AATCC Test Method. Briefly, 1.0 ml of 12 hours challenge bacterial inoculum (Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 6538) was dispersed as droplets over the 3 swatches (test fabrics) using a micropipette. The swatches were inoculated in pre-sterilized 250 ml Erlenmeyer flasks. After all the samples were inoculated, the flasks were incubated at 37 ± 2 °C for 18h before being assayed for bacterial population density. The bacterial population density was determined by extracting the bacteria from the fabric by adding 100 ml of distilled water to each flask and shaken using an orbital shaker for 1 min. Then aliquots were serially diluted and pour plated to determine the bacterial density. The difference in number of viable bacteria was evaluated on the basis of the percentage reduction test. Percentage reduction was calculated using the following formula.

R= (A-B) / A X 100

Where, R is percentage reduction; A is the number of bacteria in the broth inoculated with treated test fabric sample immediately after inoculation i.e., at zero contact time and B is the number of bacteria recovered from the broth inoculated with treated test fabric sample after the desired contact period of 18 hours.

Herbal-Microbial pigment composite mixture for finishing the fabrics and to investigate the mosquito repellent properties against *Aedes egyptii* mosquitoes. Specially designed excito repellency test chambers (Plate IX) were used to evaluate the efficiency of repellency activity.

The outer chamber of excito-repellency testing device measures 34 cm × 32 cm × 32 cm and faces the front panel with the single escape portal. The box is composed of a rear door cover, an inner transparent glass panel with a sealed door. Laboratory tests were performed during daylight hours and each test was replicated twice. After each test was completed,

the number of specimens dead and the number of specimens escaped was recorded for all the samples (before wash and after wash). The percentage of Mosquito repellency was calculated by the following formula,

$$\text{Mosquito Repellency (\%)} = \frac{\text{No: of specimens escaped + No of specimens dead}}{\text{No: of specimens exposed}} \times 100$$

The surface finishing's of the Tencel and viscose fabrics with the developed herbal-microbial pigment composite was observed using Scanning electron microscopy (SEM).

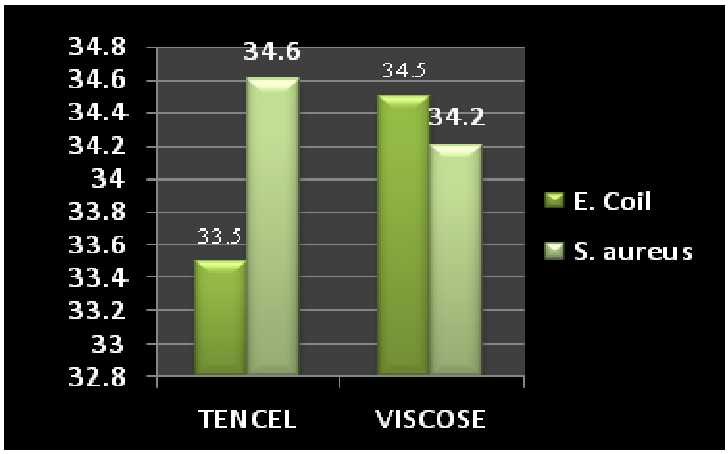
viscose fabric samples were observed using SEM. A formation of Capsule layer in white colour on the yarn is evident from the image for tensile fabric and deposition of composite as capsules in the interstices of yarn is evident from the image for viscose fabric.

RESULTS AND DISCUSSION

It has been found that *P.fluorescence* pigment is a good antimicrobial agent, as it can inhibit both gram positive and

Table 1. Antibacterial activity of pigment finished fabric Qualitative tests - Parallel streak method

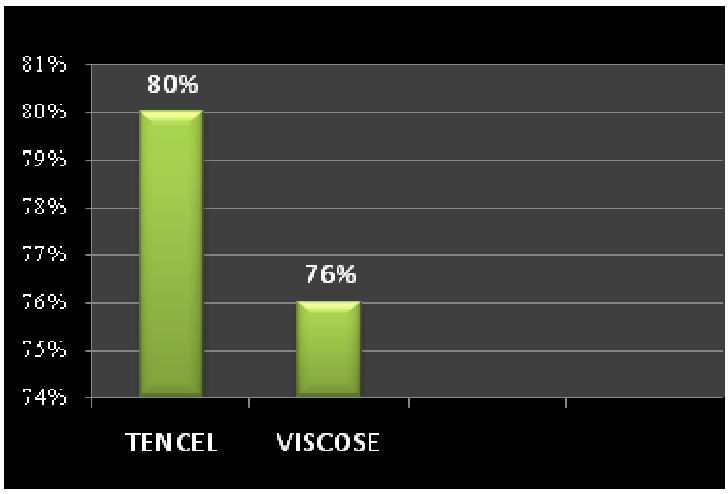
Fabric samples	*E. Coli*			*S. aureus*		
	Mean	t Value	p Value	Mean	t Value	p Value
Tencel	33.5	1.1952	0.2475	34.6	0.6928	0.4973
Viscose	34.5			34.2		



Qualitative tests - Parallel streak method

Table 2. Result of Mosquito repellence behaviour of the fabric (before and after wash)

S. No.	Fabric sample	Mosquito Repellent: Excito Chamber test method
1	100% Tencel (control)	Nil
2	100% Tencel (composite finished)	80.0%
3	100% Viscose (control)	Nil
4	100% Viscose (composite finished)	76.0%



Mosquito repellence behaviour of the fabric after wash

of the fabrics. The topographic analysis of finished and control fabric samples were prepared for SEM using a suitable accelerating voltage (10 KV), vacuum (below 5 Pa) and magnification (X 3500). Metal coating was used as the conducting material to analyse the sample. 100% Tencel and

was used for antimicrobial and larvicidal properties. Herbal plants extracts of Gliciridia sepium and pigment extract of Pseudomonas fluorescence were blended together to form a novel composite mixtures applied onto twill fabric as antimicrobial finishing agent. Tencel fabric showed notability

better repellent property than viscose twill fabric. 100% Tencel and viscose fabric samples were observed using SEM. A formation of Capsule layer in white colour on the yarn is evident from the image for tensile fabric and deposition of composite as capsules in the interstices of yarn is evident from the image for viscose fabric. Parallel streak method results clearly show that all treated fabrics are having very good antibacterial properties to both Gram positive and gram negative micro–organisms. The treated fabrics do not allow the growth of bacteria under the test specimens. All the treated fabrics show a zone of inhibition ranging 9.9 mm to 12.5 mm for Gram positive and from 4.9 mm to 6.6 mm for Gram negative bacteria. In the Larvicidal activity the result shows there was no lethal effect was observed after an hour in all the concentrations.

In Table – 1it shows that there were progressive increases in the lethal effect on the larvae after about three hours of exposure to the extract. The percent mortality and lethal concentration were calculated. During the excite chamber test, the fabric exhibited 80% of repellency for Tencel fabrics and 76% for viscose fabrics separately. In Table-2 the obtained results were evaluated using the formula given and the repellency behaviour of the fabric was presented in percentage. Composite finished fabrics (Tencel and viscose) exhibits significant repellent properties, which deserve to utilize as mosquito repellent home textile materials and equivalent.

Conclusion

The result of the tencel and viscose fabrics finished with Herbal-Microbial pigment composite mixture shows uniformly coated picture using Scanning Electron Microscopy (SEM). The research shows that the twill weaved Viscose and Tencel fabrics finished with the larvicidal activity of bacterial pigments and *Gliciridiasepium* extracts against *Aedes aegypti* larvae is highly appreciable. The present research work concludes that the result of the novel composite extraction of Gliciridia sepium and Pseudomonas fluorescence shows excellent mosquito repellence using Excito-chamber test method.

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