CHAPTER V

SUMMARY AND CONCLUSIONS

Heritage textiles have played a crucial role in the customs and traditions of people throughout history. These textiles serve as significant archives that represent human history, cultural values, and artistic creations. Traditional textiles are displayed and stored in museums all around the world, including artifacts from archaeological excavations, caskets, carpets, tapestries, and ornamental fabrics, costumes, and robes that are almost a century old. These textiles are usually made of natural fibers that can be either cellulosic or protein in nature, and they undergo deterioration easily. They are unique, fragile, and demand care, preservation, and conservation for them to survive for future generations, especially in tropical countries like India where the temperature and humidity are relatively high. Apart from the physical and chemical degradation, the main causes of biological deterioration are usually excessive moisture, warmth, and food supply, which create the ideal environment for moulds, bacteria, and pests to quickly multiply and cause discoloration, fibre breaking, decline in polymerization, holes, and ultimately complete destruction.

Traditional preservative techniques in India involve using dry herbs and spices such as cloves, cinnamon, camphor, neem leaves, tobacco leaves, tulsi, lavender, and eucalyptus, among others, to repel insects and kill bacteria. The various active ingredients found in herbs and spices such as eugenol in clove, monoterpenes in camphor, nicotine in tobacco leaves, phenolic chemicals in lavender oil, and carvacrol in oregano. These ingredients have biocidal and insect repellant effects, making them effective for preserving textiles against pests. However, the traditional techniques involve layering dried leaves beneath preserved textiles, which requires constant cleaning and inspection, and the active ingredients found in herbs and spices are frequently light-sensitive and cannot be applied directly to a surface or fabric.

To overcome these difficulties, the researcher developed a nanoparticle that were coated on cotton and polyester fabrics using a pad dry cure method. The essential oils such as neem, clove, cinnamon, carom oil, were used to formulate nanoparticles using chitosan as a polymer. This made

the oils more stable and provided biocidal and insect repellent properties to the textiles. The essential oils diffuse through the chitosan polymer shell, allowing a regulated release of the active compounds and providing long-lasting protection to the textiles against bacteria, fungi, and insects. The polymer shell also helps to prevent the active compound from oxidation and UV degradation.

The aim was to develop a preservative fabric that will provide a scientific approach to preserving heritage textiles and increasing their lifespan without directly applying any finishes onto them. The developed nanoparticle-coated fabric can be used to cover heritage textiles when in storage, as a lining for flat storage, and as padding on hangers and rollers. It can also be used as a backing or covering material for exhibits, helping to slow down the degradation processes and limit further aging of museum textiles while preserving as much as possible their unique characteristics for now and future generations. This will also help to sensitize current and future generations about traditional indigenous practices of preserving textiles. Textiles have historically played important roles in various cultures, often reserved for ceremonial or specific classes of people. Special textiles, such as quilts and heirloom textiles, hold sentimental value and can be treasured for their memories and representation of past lives. The preservative fabric developed under this study can also be used by individuals at home to preserve their personal collections and heirlooms.

To achieve the intensive exploration and experimental process, the objectives framed for the study are as follows:

5.1 Objectives of the study

5.1.1 To study and understand the preservative practices adopted by textile museums and by individuals at home.

5.1.2 To isolate and identify the microorganisms present on the deteriorated cellulosic and protein fabrics.

5.1.3 To identify the essential oils and develop nanoparticles using essential oils.

5.1.4 To study and compare the properties of nanoparticles in terms of its particle size, encapsulated efficiency, loading capacity, and retention property.

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5.1.5 To determine the minimum inhibitory concentration of the developed nanoparticles and to compare its efficacy using individual and combination of nanoparticles against the selected microbial strains.

5.1.6 Application of the optimized essential nanoparticles on the substrate and to test the microbial and insect repellency.

5.2 Experimental procedure

To achieve the objectives of the study, the research was divided into three phases:

Phase I:

The researcher conducted an extensive review and survey at several textile museums and with individuals who are textile enthusiasts with their personal textile collection to understand the preservative practices used for the textiles. Primary data was achieved by conducting interviews with textile collectors, conservators, and curators using open-ended questionnaires. Secondary data sources were also used to obtain information on textile preservation and conservation methods.

An experimental approach was taken to understand the microflora environment of a preserved cellulosic and proteinic natured textile, and the microbes on a preserved cotton, wool, and silk fabric that had undergone deterioration were identified. The study focused on isolating and identifying the microbes present on preserved cellulosic and protein textiles. Fabrics of cotton, silk, and wool that had undergone degradation were selected for the investigation. The procedure involved using sodium chloride to extract the microbes from the fabric, nutrient agar to isolate all bacteria, and potato dextrose agar medium to isolate fungi. Additional media were used to isolate and classify gram-positive and gram-negative bacteria. Biochemical tests like Catalase, and Oxidase test, and Motility test were performed to further characterize the bacterial isolates followed by growing the bacterial colonies on selective media to confirm their identification.

Phase II focused on developing the essential oil nanoparticle finish. The essential oils used were neem, clove, cinnamon, and carom, were chosen for their antimicrobial and insect repellent

properties. Chitosan was used as the wall material/shell due to its great matrix capabilities and antimicrobial and mucoadhesive properties. The nanoparticles were prepared using two methods: emulsification followed by ionic gelation and nano-emulsion followed by ionic gelation. The formulation and process parameters for developing the nanoparticles were optimized.

Different percentages of chitosan solutions (0.5, 1, 1.5, and 2%) were prepared by dissolving chitosan in acetic acid. Different polymer: oil ratios (1:1, 1:2, and 1:3) were prepared separately in 10 ml of solvent suitable for the significant essential oil. Two different types of surfactants, Tween 80 and Poloxamer, were tested in various concentrations (0.5, 0.75, 1, and 2%) for the preparation of nanoparticles using both methods. Different concentrations of the crosslinking agent, Sodium Tripolyphosphate (TPP) (1%, 2%, and 3%) were utilized to fabricate nanoparticles. The stirring rate and stirring time were also optimized.

All the formulation and process parameters were optimized on the basis of the highest entrapment efficiency obtained for each essential oil. Overall, this section provides a detailed account of the preparation and optimization of essential oil nanoparticles using chitosan as a polymer. The characterization and optimization of the developed nanoparticles was done on the basis of following methods. First, a calibration curve for the essential oils using Ultraviolet/Visible spectroscopy (UV-Vis spectroscopy) to determine the entrapment efficiency of the oils in the nanoparticles. Then determination of the encapsulation efficiency (EE%) and loading capacity (LC%) of the nanoparticles was conducted by adding a solvent suitable for the essential oil to the nanoparticles, centrifuging the solution, and separating the supernatant to estimate drug loading efficiency using (UV-Vis spectroscopy). The particle size and polydispersity index (PDI) of the nanoparticles were then analyzed using Dynamic light scattering (DLS). The minimum inhibitory concentrations (MIC) of the nanoparticles were also determined using the microdilution method and four bacterial colonies Bacillus cereus, Staphylococcus, Pseudomonas , and Escherichia coli were selected to examine the repelling properties of the developed nanoparticles under the study. The surface morphology of the nanoparticles was studied using Scanning electron microscope (SEM) analysis at various magnifications.

Two substrates were chosen to apply the optimized essential oil nanoparticles on: cotton and polyester fabric. Cotton was selected for its durability and breathability, while polyester was

chosen because it is a synthetic material that may hinder the growth of microbes. The nanoparticles made from neem, clove, and cinnamon essential oils at their optimized conditions were used for the application, along with 10% citric acid binder. The fabric samples were dipped in the nanoparticle finish for 15 minutes, passed through a padding mangle, and then cured and air-dried.

Phase III involves the assessment of the coated fabrics, specifically the evaluation of the antibacterial and antifungal activities, surface analysis using energy dispersive X-ray spectroscopy (EDX), physicochemical stability of nanoparticles over time and storage conditions, and determination of insect resistance.

To evaluate the antibacterial activity of untreated and NP-coated fabrics, the Parallel Streak Method (AATCC 147) was used. The fabrics were placed in contact with an agar surface in separate petri dishes inoculated with *Bacillus cereus*, *Staphylococcus*, *Pseudomonas*, and *Escherichia coli.* The effectiveness of the finish was determined by observing the inhibition zone around the samples. The antifungal activity was evaluated using the AATCC 30 method. *Aspergillus fumigatus* was grown on a solid medium, and a spore suspension was used to inoculate an agar medium in a petri dish. The test samples were placed on top of the inoculated agar medium, and then the top of the sample was inoculated with the spore suspension. The inoculated test samples were evaluated and rated based on the presence of macroscopic, microscopic, or no growth.

EDX analysis was used to measure the elemental composition of the nanoparticle treated cotton and polyester samples to confirm the presence/ coating of the finish on to the fabric. The physicochemical stability of the nanoparticles over time and storage conditions was also conducted. The experiment aimed to determine the changes in the amount of oil trapped in nanoparticles over a period of two months under different storage conditions to have an understanding of the percent release and percent retention of the oil from the nanoparticles. The finished nanoparticle cotton fabrics were evaluated for one and two months, with one set of samples kept in a closed environment in a petri dish, and the other set exposed to an open environment. Further, the resistance to insects was evaluated using the ISO 3998:1977 standard

by testing treated cotton fabric samples with neem, clove, cinnamon, and a combination of clove and cinnamon essential oil chitosan nanoparticles for their repellency against cigarette beetles.

5.3 Results and Discussions

5.3.1 Studying the preservative practices adopted by textile museums and by individuals at home.

After visiting seven museums, researcher observed that all the museums had used incandescent and LED lights to emit low levels of ultraviolet and blue light to reduce the negative impact of light on textiles, as well as ceiling fans to maintain a consistent temperature and air circulation to prevent heat and humidity buildup, which can damage textiles. Exhaust fans had also been used to remove excess moisture and pollutants from the air, which can cause mold growth and discoloration of fabrics.

Textile collectors had also taken steps to preserve their textiles. According to an open-ended questionnaire with six textile collectors, the greatest threats to textiles were dust, light, insects, microorganisms, temperature, and relative humidity. Respondents stored their textiles in metal trunks and wooden wardrobes, wrapped them in washed mull fabric before storage, and minimized the number of folds when storing. Most respondents changed the folds of their textiles yearly, and some exposed the textiles to sunlight for a few hours once a year to aerate and kill microbes.

The most common preservative observed in museums and by textile collectors was the use of natural herbs and spices. Tobacco leaves, neem leaves, cloves, cinnamon, lavender, and sandalwood were all used to protect textiles from insects and microbes. Naphthalene balls are also commonly used but can leave behind stains on fabrics and textiles. Carom seed oil and dried cannabis leaves are suggested as alternatives. However, traditional preservation methods may have limitations and may not be effective or practical for preserving large collections of heritage textiles over extended periods. They may not work on all types of pests or may only work for a short time. Also, sachets are usually small, so they may not cover larger textiles completely. Insects and microbes may still be able to get into areas that are not covered by the sachet. Furthermore, some

herbs and spices may have pigments or oils that can stain textiles, especially delicate or valuable ones. Using a bed of dried neem and tobacco leaves beneath textiles can be a time-consuming task and may also cause damage to the textiles if the leaves are contaminated with small particles.

To address these limitations, the researcher approached the method of combining the traditional practices with a more scientific approach to develop a preservative fabric that provides a better preservation environment over a larger surface area for a longer time, resulting in a preservation method that is effective, practical, and has no adverse side effects. Based on the review and the interacting between the museum conservators, essential oils like neem, clove, cinnamon, and carom were selected to develop the finish.

5.3.1 To isolate and identify the microorganisms present on the deteriorated cellulosic and protein fabrics.

The study analyzed cotton, silk, and wool fabrics that were over 60 years old had undergone preservation with naphthalene balls, and wool that had undergone moth infestation and later exposed to naphthalene balls. The objective was to identify the microorganisms that had grown on these fabrics and caused their deterioration. The microorganisms were isolated and identified through various morphological and biochemical tests. *Bacillus cereus* species were identified as the dominant Gram-positive bacteria on all three fabrics followed by *Staphylococcus aureus*, while *Pseudomonas* species were the dominant Gram-negative bacteria found on all three fabrics. The number of Gram-positive bacteria exceeded the number of Gram-negative bacteria in all three types of fabric. The cotton fabric had the highest number of colonies, followed by silk and then wool fabric. *Aspergillus* was the dominating fungal colony observed on the deteriorate textiles. The study chose the dominating species isolated from the deteriorated fabrics *Aspergillus, Bacillus cereus, Staphylococcus aureus, Pseudomonas*, along with *Escherichia coli*, which is a reported bacteria that damages textiles, to test the effectiveness of the newly developed finish in repelling these colonies.

5.3.2 Developing nanoparticles using essential oils and optimizing them for its highest entrapment efficiency and loading capacity.

The preparation of essential oil chitosan nanoparticles was done using two different methods, the nano-emulsion+ Ionic gelation method and emulsion+ ionic gelation method. Different essential oils such as neem, clove, cinnamon, and carom were selected as a core material. Chitosan was used to encapsulate the essential oil due to its biodegradability, cationic charge, and muco-adhesive nature. Different percentages of chitosan solutions and polymer: oil ratios were prepared, and two surfactants, Tween 80 and Poloxamer 188, were tested in various concentrations for the preparation of nanoparticles. Different concentrations of the crosslinking agent, Sodium Tripolyphosphate (TPP), were used to fabricate nanoparticles, and the stirring rate and stirring time were optimized. The highest entrapment efficiency of the essential oil in the nanoparticles was achieved by optimizing the amount of chitosan, essential oil, surfactant, TPP concentration, stirring rate, and method of nanoparticle preparation.

- Poloxamer 188 was chosen for cinnamon nanoparticles, while Tween 80 was selected for neem, clove, and carom essential oil nanoparticles. The nano-emulsion+ ionic gelation method was chosen for neem oil chitosan nanoparticles, while the emulsion+ ionic gelation method was selected for clove, cinnamon, and carom essential oil nanoparticles.
- **Chitosan:** The concentration of chitosan was adjusted to 0.5%, 1%, 1.5%, and 2%, while surfactants were maintained constant. The highest entrapment efficiency was observed at 1% chitosan concentration for all essential oils. The increase in entrapment efficiency was attributed to the increase in the available surface area for interaction with TPP, resulting in smaller particles with a higher surface area-to-volume ratio. However, when chitosan concentration was further increased beyond 1%, entrapment efficiency decreased due to the formation of larger particles or aggregates, reducing surface area available for interaction with TPP. Loading capacity varied with entrapment efficiency, with an increase in entrapment efficiency causing an increase in loading capacity. The nanoparticles' size was observed to be higher at 0.5% chitosan than at 1%, potentially due to insufficient chitosan available to form a wall layer for all essential oils. 1% chitosan was considered the optimized condition for the nanoparticles.

- **Surfactant**: Tween 80 was more effective in developing neem, clove, and carom essential oil nanoparticles, while poloxamer 188 worked better for cinnamon oil nanoparticles. Both surfactants reduced the interfacial tension between the oil and aqueous phase, leading to improved entrapment efficiency. The study found that the optimal concentration of Tween 80 for neem, clove, and carom essential oil nanoparticles was 0.75%, with a decrease in efficiency observed at higher concentrations. In contrast, the optimal concentration of poloxamer 188 for cinnamon essential oil nanoparticles was 0.50%, with a decrease in efficiency at higher concentrations. The excess surfactant may have disrupted the droplet structure, leading to decreased encapsulation efficiency. The surfactant concentration also played a significant role in controlling the size of nanoparticles, with smaller particles observed at the optimal concentration of 0.75% Tween 80 and larger particles observed at higher concentrations. The excess surfactant may have accumulated and formed micelles, leading to larger nanoparticle sizes.

- **Oil:** The entrapment efficiency and loading capacity of oil-loaded nanoparticles were affected by the concentration of oil used in the encapsulation process. The study showed that the highest entrapment efficiency was observed at 1% oil concentration for all the essential oils tested. However, as the oil concentration increased to 2% and 3%, the entrapment efficiency decreased drastically in all the nanoparticles due to the oil droplets becoming more difficult to encapsulate within the nanoparticle matrix. This decrease in efficiency can be attributed to the likelihood of oil droplets coalescing or merging, leading to larger droplets that are more challenging to encapsulate.

  The loading capacity of nanoparticles increased with the increase in percentage of oil in all the essential oil chitosan nanoparticles. However, the entrapment efficiency decreased as the oil content increased. This could be due to the saturation of essential oil loading into the wall material during the encapsulation process.

  The percentage of oil used in the formulation also significantly impacted the size of the resulting nanoparticles. The size of the nanoparticles generally tends to increase with the increase in oil percent due to the coalescence of oil droplets during the emulsification and gelation process.

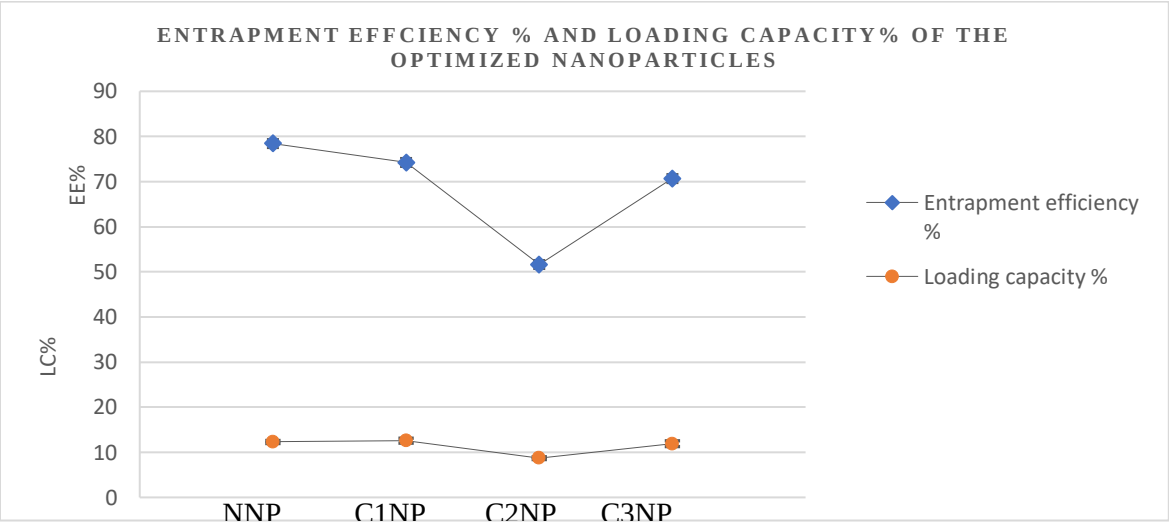
- **TPP:** The use of TPP as a crosslinking agent to create chitosan nanoparticles with essential oils. The interaction between the negatively charged TPP and positively charged chitosan results in the formation of a gel-like structure that entraps drug molecules within the nanoparticle matrix, improving stability, controlling size, and enhancing entrapment efficiency. The study found that the highest entrapment efficiency and loading capacity was observed in neem nanoparticles, followed by cinnamon oil, carom oil, and clove nanoparticles, when 1% TPP solution was added. However, as the concentration of TPP increased beyond a certain point (2% and 3%), the encapsulation efficiency decreased significantly in all nanoparticles. The loading capacity of essential oil nanoparticles was highest at 1% TPP, regardless of the type of oil used. The size of nanoparticles is mainly influenced by the concentration of TPP in the chitosan solution, with smaller nanoparticles formed at 1% TPP due to increased stability, while larger nanoparticles formed at higher TPP concentrations due to larger chitosan-TPP complexes.

- **Stirring rate (RPM):** The stirring rate is important for nanoparticle preparation, affecting particle size and entrapment efficiency. For emulsification and ionic gelation, an optimized stirring rate of 1800 rpm resulted in high entrapment efficiency and loading capacity, and smaller particle size. Cinnamon oil chitosan nanoparticles had the highest entrapment efficiency and loading capacity. A two-stage process involving high shear homogenization at 13,000 rpm was optimized for neem essential oil chitosan nanoparticles followed by stirring at 1800 rpm which resulted in smaller particle size and higher entrapment efficiency. Neem essential oil nanoparticles had the highest entrapment efficiency and loading capacity amongst all oils. Further increase in homogenization speed decreases entrapment efficiency and loading capacity. High shear can cause essential oils to evaporate and result in particle aggregation.

The optimized conditions for all the essential oil chitosan nanoparticles are represented in the below table 5.1

Table 5.1: Optimized conditions of the final nanoparticles

Optimized conditions of the essential oil nanoparticles						**Results**	
Oil	Method of preparation	Polymer: Oil	Surfactant %	TPP %	RPM	Size (nm)	PDI
Neem	Nano-emulsion+ Ionic gelation	1:1	0.75 (Tween 80)	1	13000 for 10 min followed by 1800	189 ± 26	0.364
Cinnamon	Emulsion+ Ionic gelation	1:1	0.5 (Poloxamer 188)	1	1800	226 ± 25	0.241
Clove	Emulsion+ Ionic gelation	1:1	0.75 (Tween 80)	1	1800	294 ± 33	0.287
Carom	Emulsion+ Ionic gelation	1:1	0.75 (Tween 80)	1	1800	349 ± 23	0.450



Graph 5.1: Entrapment efficiency % and Loading capacity % of all the four optimized nanoparticles

5.3.3 Characterization of the developed nanoparticles

5.3.3.1 Calibration curve of essential oils:

Several standard solutions were prepared by diluting the standard compound in an appropriate solvent, and the resulting solutions were analyzed for their absorbance using a UV-Vis Spectrophotometer. Clove essential oil was dissolved in dichloromethane, while carom essential oil was dissolved in methanol. Cinnamon essential oil was dissolved in a mixture of 70% methanol and 30% water, and neem essential oil was dissolved in a mixture of 54% dichloromethane and 46% methanol. The absorbance spectrum of neem essential oil showed a peak at 278nm, cinnamon oil at 287 nm, clove oil at 278 nm, and carom at 277nm. A linear calibration curve was created by plotting the concentration of the standard solutions on the x-axis and the absorbance on the y-axis. The calibration curve equation was generated by calculating the regression coefficient (R2). The percent entrapment efficiency and loading capacity of the nanoparticles were determined by using the calibration curve of essential oils as a standard reference point for measuring the amount of oil encapsulated within the nanoparticles. The regression coefficient for neem oil was $y = 0.0007x - 0.049$ with $R^2 = 0.9942$, for cinnamon oil was $y = 0.1409x + 0.061$ with $R^2 = 0.9956$, for clove oil was $y = 0.0224x - 0.0117$ with $R^2 = 0.9975$, and for carom oil was $y = 0.0078x + 0.0852$ with $R^2 = 0.994$.

5.3.3.2 Size and PDI: As observed in table 5.1, the results showed that the neem chitosan nanoparticles had the smallest size of 189 nm, while the cinnamon chitosan nanoparticles had a slightly larger size of 226 nm, and the clove chitosan nanoparticles had the largest size of 294 nm. In terms of size distribution, the cinnamon chitosan nanoparticles had the narrowest size distribution with a PDI of 0.241, while the neem chitosan nanoparticles had a higher PDI of 0.364, indicating a broader size distribution. The clove chitosan nanoparticles had the widest size distribution with a PDI of 0.287. The PDI values provide an indication of the uniformity of particle size distribution, with a lower PDI value indicating a more uniform particle size distribution. The values obtained for all three types of optimized nanoparticles were relatively low, indicating that the particle size distributions were relatively narrow.

5.3.3.3 Minimum Inhibition Growth (MIC): MIC of the four developed nanoparticles along with neem +clove and cinnamon+ clove in combination was calculated against two gram-positive bacteria, Bacillus cereus and Staphylococcus aureus, as well as two gram-negative bacteria, Pseudomonas and E. coli, and a fungus, Aspergillus fumigatus. The results showed that all the nanoparticle samples had broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria, but there were differences in the MIC values for the different types of nanoparticles. Clove essential oil nanoparticles (C2NPs) were found to be the most effective at inhibiting bacterial growth at a lower concentration compared to other single essential oil nanoparticles, and combining Cinnamon and Clove nanoparticles (C1NPs+C2NPs) enhanced their antimicrobial activity. However, Carom essential oil nanoparticles (C3NPs) did not exhibit any antibacterial or anti-fungal activity, despite having a high entrapment efficiency at any dilution, except at the undiluted concentration. Hence, Carom oil was not taken further in the application. The study also found that a higher concentration of nanoparticles was needed to inhibit the growth of gram-negative bacteria compared to gram-positive bacteria, which could be attributed to the differences in their cell walls. The differences in MIC values for the different types of nanoparticles were likely due to differences in the types and concentrations of bioactive compounds present in the essential oils used to prepare the nanoparticles. Overall, the study suggests that essential oil chitosan nanoparticles have potential as antimicrobial agents, especially when used in combination.

5.3.3.4 SEM analysis: The SEM images of neem, cinnamon and clove essential oil nanoparticles showed that they had a spherical and uniform shape, suggesting that the synthesis process was consistent. The particles' size observed using the SEM images are similar to the size measured using a Zeta sizer. Moreover, the particles do not have any cracks, indicating that the wall layer formed continuously during the synthesis process. However, a few particles showed inward grooves or dents, which could have resulted from the diffusion of oil or the lyophilization process.

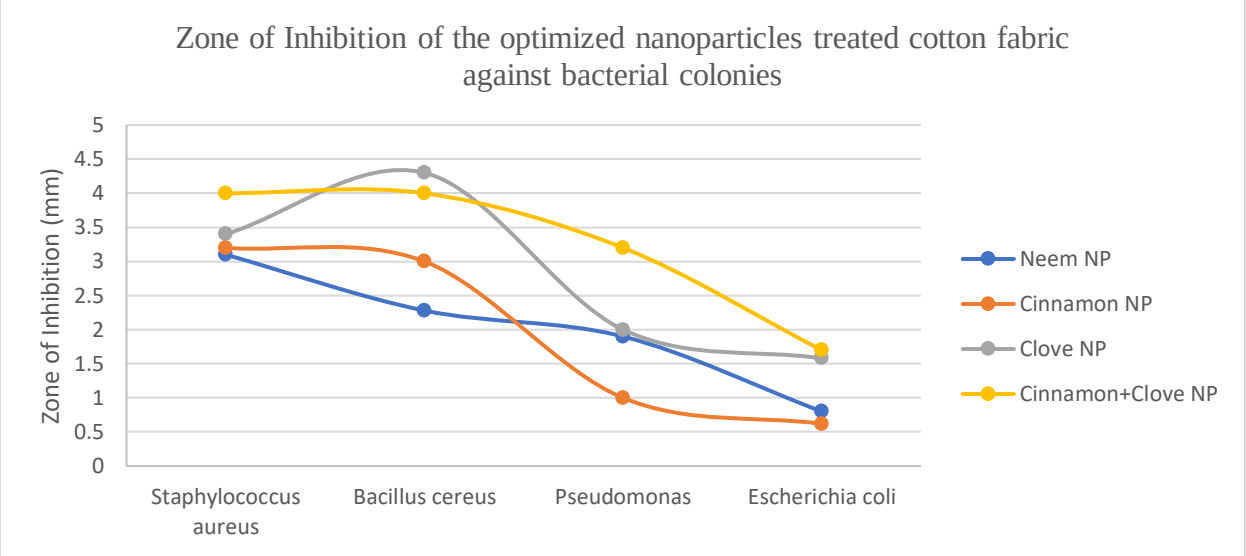
5.4 Application of the optimized essential nanoparticles on the substrate

The study selected neem, clove, cinnamon, and a combination of clove and cinnamon essential oil nanoparticles for application based on their minimum inhibitory concentration (MIC) results. The optimal solution was determined by diluting the nanoparticles to the desired concentration that

obtained from the results of MIC for coating the cotton and polyester substrate. The optimized solution was coated on the fabric samples using a padding mangle, along with a 10% citric acid binder which also acts as a dispersing agent. The fabric samples were dipped and padded using a padding mangle in the nanoparticle solution followed by drying and curing.

5.5 Assessment of the treated fabric

5.5.1 Assessment of the antibacterial (AATCC 147) and antifungal activity (AATCC 30):



Graph: 5.2: Zone of inhibition (mm) of the optimized nanoparticle treated cotton fabric against the selected bacterial colonies

The test investigated the efficacy of essential oil-loaded chitosan nanoparticles and blank chitosan nanoparticles against four different bacterial strains on cotton and polyester fabrics. The results indicated that the hydrophobic nature of polyester hindered the absorption of the finish and interaction of nanoparticles with bacterial cells, leading to no inhibition of all tested bacterial strains on polyester fabric, regardless of the type of nanoparticle treatment used. The study found that the effectiveness of oil nanoparticles against different bacterial species varied solely depending on the type of oil used in the nanoparticle and the fabric substrate while blank chitosan nanoparticles had no effect on bacterial repellency. As shown in graph 5.1, among the four essential oil nanoparticles tested, a combination of clove and cinnamon oil nanoparticles showed the highest zone of inhibition against *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas*, and

E. coli. Clove essential oil nanoparticles which contain high concentration of eugenol exhibited the strongest antibacterial properties, despite having the lowest entrapment efficiencies compared to other essential oil nanoparticles. Despite of the ability of the bacterial repellency, neem oil nanoparticles consistently showed the lowest zone of inhibition against all bacterial species tested which might be the result of its slow-release mechanism explained in the retention study.

Over all, the study suggested that gram-positive bacteria may be more susceptible to essential oils compared to gram-negative bacteria. In conclusion, the study suggests that clove and cinnamon oil nanoparticles, particularly in combination, showed a synergetic broad-spectrum antimicrobial effect against different bacterial species causing the highest zone of inhibition of 4mm.

The effectiveness of chitosan nanoparticles in preventing fungal growth on cotton and polyester fabrics was also investigated against Aspergillus fumigatus. The results showed that cotton fabric treated with chitosan nanoparticles was somewhat successful in repelling fungal growth, while polyester fabric treated with chitosan nanoparticles did not show any repellency. This was attributed to the hydrophobic nature of polyester, which made it difficult for the chitosan nanoparticles to adhere to the fabric surface, resulting in less effective absorption.

5.5.3 EDX analysis

As the antimicrobial study found that cotton fabric was effective in repelling microbes, while polyester fabric did not have any repellent properties which could be due to the hydrophobic nature of polyester fabric, which hindered the absorption of the finishing agent. EDX analysis was conducted to investigate this further, and the results showed that essential oil nanoparticles were successfully applied to the cotton fabric, as apart from the presence of carbon and oxygen, nitrogen, phosphorus, sodium was observed on the treated cotton fabric indicating the presence of chitosan and TPP from the nanoparticles while no presence of minerals apart from carbon and oxygen were present on any of the samples of polyester fabric. This lack of presence of elements could be attributed to the hydrophobic nature of polyester fabric, which inhibits the absorption of the finish onto the fabric. Based on these findings, it was concluded that cotton fabric was the optimal substrate, and only treated cotton fabric was used for further tests.

5.5.4 Determining the physicochemical stability of the nanoparticles over time and storage conditions

The study aimed to evaluate the effectiveness of fabric treated with essential oil nanoparticles in different environmental conditions. Samples were exposed to closed and open environments and the entrapment efficiency was calculated to determine the percentage of oil retained in the nanoparticles after one and two months. The data showed that the fabric treated with neem essential oil loaded nanoparticles exhibited the highest oil retention of 99.10% after one month and 97.32% after two months in a closed environment, followed by clove oil which exhibited the highest oil retention of 98.56% after one month and 97.13% after two months in a closed environment but not in an open environment. Cinnamon oil had the second-highest release rate in an open environment. Neem essential oil exhibited the highest oil retention and this slow-release rate of the oils was found to affect their antibacterial activity, with slower release rates resulting in lower activity. The effectiveness of the treatment decreased over time as the release rate increased, but the percentage of essential oil trapped in the nanoparticle remained above 50% even after exposure to the environment for two months, indicating the durability of the treated fabric.

5.5.5 Determination of resistance to insects using standard ISO 3998:1977

Cigarette beetles, also known as Lasioderma serricorne, are reported to harm textiles stored in museums. They feed on the fibers which can lead to damage such as holes and tunnels. This makes them especially dangerous for ethnographic collections since they reproduce quickly. The repellency of the larvae of Cigarette beetles were tested against the treated cotton fabric with optimized conditions, including neem essential oil loaded chitosan nanoparticles, clove essential oil loaded chitosan nanoparticles, cinnamon essential oil loaded chitosan nanoparticles, and a combination of clove and cinnamon essential oil loaded chitosan nanoparticles. The developed essential oil chitosan nanoparticle treated cotton fabric were The experiment found that all larvae in the treated fabric jars moved away from the fabric and eventually died, while some larvae were still on untreated fabric samples, and others had died due to a lack of nutrition. There was no damage observed on any treated or untreated fabric samples, and there was no loss in weight. The results suggested that the essential oil loaded chitosan nanoparticles in the treated fabric samples

were successful in repelling the larvae and causing their demise through prolonged exposure to volatile compounds. The use of essential oil loaded chitosan nanoparticles treated cotton fabric could potentially prevent insect and pest infestations.

Conclusion

- It was found that both museums and textile collectors use various methods to preserve textiles, including controlling light, temperature, humidity, and using natural herbs and spices to protect against insects and microbes. However, traditional preservation methods may have limitations and may not be practical for large collections of heritage textiles over an extended period.
- For the proposed study, use of essential oils such as neem, clove, cinnamon, and carom were selected to develop a finish that provides a better preservation environment over a larger surface area for a longer time, resulting in a preservation method that is effective, practical, and has no adverse side effects.
- The dominant bacteria isolated from a 60-year-old preserved cotton, silk and wool fabric were Bacillus cereus and Staphylococcus aureus, while Pseudomonas species were the dominant Gram-negative bacteria and Aspergillus was the dominating fungal colony observed on the deteriorated textiles. The study selected these dominant species isolated from the deteriorated fabrics along with *Escherichia coli* to test the effectiveness of the newly developed finish in repelling these colonies.
- Neem, cinnamon, clove and camphor essential oil chitosan nanoparticles were prepared using two different methods, the nano-emulsion+ Ionic gelation method and emulsion+ ionic gelation method.
- The size of the nanoparticles developed ranged from 189- 350 nm, and their size distribution was found to be uniform with PDI ranging between 0.241-0.450.
- The highest entrapment efficiency and loading capacity were observed in neem and cinnamon oil nanoparticles with 78.42 ± 1.56% and 74.23 ± 2.11% and 12.28 ± 0.34% and 12.57 ± 0.55%, respectively followed by carom oil with entrapment efficiency of 70.66 ± 2.24% and loading capacity of 11.9 ± 0.64%. On the other hand, clove oil nanoparticles

had the lowest entrapment efficiency and loading capacity of 51.62 ± 3.1% and 8.7 ± 0.31%, respectively.

- The MIC results showed that all the nanoparticle samples had broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria with Clove essential oil nanoparticles (C2NPs) to be the most effective at inhibiting bacterial growth at a lower concentration compared to other single essential oil nanoparticles, and combining Cinnamon and Clove nanoparticles (C1NPs+C2NPs) enhanced their antimicrobial activity.
- MIC results of Carom essential oil nanoparticles (C3NPs) did not exhibit any antibacterial or anti-fungal activity, despite having a high entrapment efficiency. Hence, carom essential oil nanoparticles were not carried forward in the study.
- The SEM images of neem, cinnamon and clove essential oil nanoparticles showed that they had a spherical and uniform shape, with absence of cracks, indicating that the wall layer formed continuously during the synthesis process.
- The developed finished was coated on the cotton and polyester fabric selected under the study with the help of a padding mangle using dipping and pad-dry-cure method.
- The antimicrobial studies found that the nanoparticles possess good antibacterial and insect repellent properties and somewhat repellency towards fungi, and can be coated on cotton fabric.
- Clove oil nanoparticle and combination of clove and cinnamon essential oil nanoparticles had the highest antibacterial effect against all the bacterial species, despite clove having the lowest entrapment efficiency.
- The study also found that nanoparticles showed high zone of inhibition towards gram-positive bacteria, when compared to gram-negative bacteria, which could be attributed to the differences in their cell walls.
- The nanoparticles did not show any repellency towards microbes when treated on polyester fabric, possibly due to the hydrophobic nature of polyester which was concluded by performing EDX analysis of the treated fabric as no presence of minerals was observed on polyester unlike cotton fabric which showed presence of sodium, phosphorus, nitrogen and even calcium.

- Neem essential oil loaded chitosan nanoparticles showed the highest oil retention after one and two months in a closed environment, while clove oil exhibited the lowest oil retention in a closed environment after one month and in an open environment after two months.
- The slow-release rate of the oils was found to affect their antibacterial activity, with slower release rates resulting in lower activity. However, even after exposure to the environment for two months, the percentage of essential oil trapped in the nanoparticle remained above 50%, indicating the durability of the treated fabric.
- The developed essential oil chitosan nanoparticle treated cotton fabric successfully repelled and caused the demise of Cigarette beetle larvae through prolonged exposure to volatile compounds without causing any damage or loss in weight of the exposed fabric.
- The developed preservative fabric can be used to cover heritage textiles when in storage, as a lining for flat storage, and as padding on hangers and rollers. It can also be used as a backing or covering material for exhibits, helping to slow down the degradation processes and limit further aging of museum textiles while preserving as much as possible their unique characteristics for now and future generations.