Novel antimicrobial finishing of synthetic horse blanket textiles using Ag zeolites: Advancing sustainable solutions for insect bite hypersensitivity management in equines

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ABSTRACT

Insect Bite Hypersensitivity (IBH) affects approximately 12% of equines in the UK and up to 60% in certain regions worldwide, presenting significant challenges for horse owners. Currently, IBH management often uses synthetic horse blankets. However, these blankets have limitations; they may not effectively prevent insect bites and do not inhibit bacterial growth in wounds associated with IBH. This raises the need for innovative, sustainable solutions that challenge conventional ideas about existing horse blanket materials. An assessment of three commercial horse blankets was conducted to analyse their designs and structures according to British Standard protocols. The findings guided the development of a novel natural fibre blend for horse blanket design. Additionally, two types of zeolites were utilised: synthetic zeolite beta (BEA) (ammonium, SiO2/Al2O3 = 25, Alfa Aesar) and natural zeolite clinoptilolite (HEU) (Grade 0.7 – 1.6 mm, ZeoClin®). Silver (Ag) was introduced into the blanket samples through ion exchange using AgNO3 (Alfa Aesar) solutions. The study evaluated the antimicrobial efficacy and durability of the Ag zeolites. The antimicrobial effectiveness of 1 cm^2 coupons from the blankets was tested against specific bacteria associated with IBH, including *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Acinetobacter baumannii*. After 12 months of outdoor exposure, the effectiveness of treated samples was re-evaluated. Results indicated that samples treated with Ag zeolites effectively inhibited bacterial growth within 24 hours. In contrast, control samples without Ag treatment showed significant growth. The Ag-modified textiles demonstrated comparable antimicrobial efficacy even after 12 months of weathering, indicating the success of the Ag zeolite modification in providing long-lasting bactericidal properties. The core innovation lies in the incorporation of antimicrobial properties using Ag zeolites, representing a novel approach to horse blanket textiles aimed at alleviating the symptoms of IBH.

Keywords: Antimicrobial Textiles, Ag Zeolites, Equine Health, IBH.

INTRODUCTION

Since their numerous applications in daily life, textiles with antimicrobial characteristics have been the focus of extensive research in recent years. These applications span sectors such as medicine and healthcare, sportswear, clothing and footwear, furniture and upholstery, air and water purification systems, food packaging, and veterinary care. The antimicrobial capability of these textiles is designed for their effectiveness in killing or inhibiting the growth of microorganisms, including bacteria, fungi, and viruses. In veterinary applications, antimicrobial textiles, especially medical dressings, are increasingly used to treat and manage various conditions, including skin infections. This focus on antimicrobial functionality has resulted in the development of textiles that offer protection and promote healing and hygiene by reducing the presence of harmful microbes.

The potential for antimicrobial textiles is especially relevant in the context of IBH, which affects a significant portion of the equine population. Throughout the history of veterinary medicine, extensive research has been carried out to identify and understand IBH as an allergic reaction to biting midge's genus *Culicoides*. The condition leads to skin lesions, irritation, and discomfort, compromising the quality of life of affected horses. Despite the widespread use of outdoor protection blankets (all made from synthetic materials), these blankets fall short of addressing both insect protection and the risk of bacterial proliferation in wounds caused by IBH-related itching. Integrating antimicrobial agents into textiles designed for managing IBH could transform animal welfare in this context. These specialised textiles would not only provide a barrier against insects but also reduce bacterial load, thereby preventing secondary infections and promoting better skin and overall health. Although antimicrobial textiles are emerging in the equestrian industry, they have not yet matched the trends seen in other sectors, with an increasing demand for multifunctional textiles. This demand is particularly notable due to the growing emphasis on sustainable solutions and circularity within the textile industry.

This study presents a new approach to equine blanket design by integrating antimicrobial features to combat secondary infections linked to IBH. It explores the use of Ag zeolites in horse blanket fabrics, a technology not previously applied in this field. Silver ions are known for their strong antimicrobial properties, which disrupt bacteria and fungi, while zeolites enhance this effect by slowly releasing silver ions. The study aims to alleviate IBH symptoms by preventing bacterial colonisation and improving the durability of the blankets through the moisture and odour absorption of zeolites. The potential benefits include better hygiene, reduced odour, and a lower incidence of secondary infections, ultimately leading to decreased healthcare costs and a reduced need for veterinary care. Key questions include the effectiveness of current commercial horse blankets against bacterial infections in IBH-affected horses, the antimicrobial potential of Ag zeolites when integrated into horse blanket fabrics, the durability of the modified textiles under different environmental conditions, as well as the potential of an alternative novel natural fibre. This study evaluated three current horse blankets with varying points of price and performance qualities for managing IBH: sample 1, a premium sample known for high-quality materials; sample 2, a mid-range sample balancing quality and affordability; sample 3, a budget-friendly option. Each sample was assessed for durability, protection, and suitability for equine use, focusing on insect bite prevention and bacterial growth management. A novel textile blend of natural fibres was developed as an alternative to synthetic fabrics, promoting more sustainable disposal and recyclable options.

AIM

Establish a technical textile specification in horse blanket design that ensures optimal protection and IBH wound management for horses. Focus on implementing Ag zeolites to provide an antimicrobial finish to the selected fabrics and developing a natural fibre alternative.

OBJECTIVES

- **Objective 1**: Analyse the composition and effectiveness of three commercially available horse blankets, focusing on their ability to manage IBH-related complications.
- **Objective 2**: Introduce Ag zeolites into the fabric of horse blankets and evaluate their bactericidal properties against microorganisms known to cause secondary infections in IBH-affected horses.
- **Objective 3**: Assess the durability of the antimicrobial properties of these textiles after twelve months of weathering.
- **Objective 4**: Develop a novel natural fibre blended textile as a sustainable alternative to IBH management horse blankets promoting sustainability in equine apparel.

LITERATURE REVIEW

Introduction to IBH:

IBH, also known as "sweet itch" or "*Culicoides* hypersensitivity," is a significant equine health issue worldwide (Cox & Stewart, 2023). The condition is triggered by allergic reactions to insect bites, primarily from the female midge (*Culicoides*) and the female black fly (Simuliidae) family, which introduce saliva into the equine skin during feeding (Cox & Stewart, 2023; Pilsworth & Knottenbelt, 2004). The immune system's overreaction to these insect bites leads to the release of histamines, causing intense itching, inflammation, and hair loss (Baker & Quinn, 1978; Fadok & Greiner, 1990). Lesions typically appear on areas such as the ears, mane, tail, and ventral midline but can become more widespread in severe cases (Cox & Stewart, 2023). Clinical manifestations include pruritus, erythema, alopecia, and crusting (Onyiche *et al*., 2022). As the condition progresses, the skin thickens, leading to chronic dermatitis and fibrosis (Anderson *et al*., 1988). The self-inflicted trauma caused by scratching often leads to secondary infections and can cause substantial discomfort for the affected equines, impacting their overall health and well-being (Pilsworth & Knottenbelt, 2004). The seasonal nature of IBH, with symptoms peaking during the breeding seasons of insects, can result in prolonged suffering. Symptoms often persist for months and worsen over time (Cox & Stewart, 2023). While traditionally regarded as a seasonal condition, IBH is increasingly recognised as a year-round issue in regions where biting insects occur throughout the year (Tugwell, 2020). The prevalence of IBH varies by region, with figures ranging from up to 12% in Great Britain to 60% in Queensland and Australia (Cox & Stewart, 2023; Riek, 1953; Schaffartzik *et al*., 2012). The condition most commonly first appears in horses at ages four to five, and it is during this time that it is typically noticed. Its effects can be physically distressing for the animals and financially burdensome for their owners.

Management Strategies for IBH:
Protective horse blankets reduce exposure to insect bites by physically separating the insect from the horse's skin (Cox & Stewart, 2023). The most suitable clothing is a lightweight rug that covers the horse's body, the neck and the belly (Marsella *et al*., 2023). Covering a horse's body entirely is challenging and impractical in some locations (particularly in regions with high temperatures and humidity). However, in a study undertaken by Marsella *et al*. (2023) in Far North Queensland, fly sheets were found to reduce clinical signs in 60% of affected horses. Most of these horses experienced little or no pruritus compared with the previous season when not using fly sheets[1]. However, there is a gap in addressing the integration of antimicrobial properties into these textiles. Most studies focus on insect-repelling or UV-blocking capabilities. Sample 1, the first of three evaluated horse blankets, illustrated in Figure 1, is a protection blanket designed to provide extensive coverage over a horse's body. This coverage is essential for preventing insect bites, which can lead to conditions such as IBH (Itchy Horse, 2021). However, the blanket does not extend to cover the legs, a decision grounded in the understanding of equine behaviour and safety. Covering a horse's legs with fabric can lead to significant risks; the horse's natural movements, such as kicking, stretching, and any gait can cause entanglement, potentially leading to severe injuries, including tendon damage (Myers, 2005).

[1] In the equestrian industry, horse blankets are referred to as ('rugs') or ('sheets'). In this study they are referred to as horse blankets for the purpose of unilateral understanding.

Figure 1*.* (sample 1) The Boett Blanket*,* Itchy Horse[2] (2021)

In response to the need for a circular and more sustainable industry, the equestrian sector has witnessed technological advancements in the design and production of horse blankets in recent years. Although these blankets still use synthetic materials, advanced weaving techniques and the integration of high-tech fibres have resulted in products that are lighter, stronger, and more effective at regulating temperature. Features such as breathable waterproof membranes, thermal reflective linings, and reinforced stitching improve the functionality and durability of modern horse blankets (The Tech Equestrian, 2022). However, advancements in blanket design to manage IBH have not been addressed. Additionally, horse blanket textiles, typically made from synthetic materials such as polyester and nylon, do not effectively prevent bacterial infections that result from skin lesions caused by itching (Westgate *et al*., 2010; Wagner *et al*., 2009). While synthetic textiles offer water resistance and UV protection benefits, they often fail to address the microbial complications associated with IBH. Studies have shown that these materials are prone to bacterial colonisation, exacerbating the condition by creating a breeding ground for pathogens (Rendle, 2014; Westgate *et al*., 2010). Other traditional management strategies for IBH include environmental controls such as using insect repellents and managing insect breeding sites, topical treatments and homemade remedies (Cox & Stewart, 2023; Tugwell, 2020).

Natural Fibre Development:
In addition to synthetic textiles, natural fibres such as Bamboo, Tussah Silk, and Blueface Leicester wool have been investigated for their environmental benefits and comfort in the context of IBH management. These fibres are biodegradable, breathable, and less likely to cause skin irritation compared to synthetic alternatives

[2] Permission granted by Alison Price, Itchy-Horse Company Ltd, Glan Y Gors, Alltami Road, Sychdyn, Mold, Flintshire

(Azam *et al*., 2023). While these natural fibres have been explored for their comfort and individual benefits, there is limited research on their combined use for IBH management or Ag zeolite modification. This study developed an innovative approach by blending these fibres into high-performance textiles, which not only enhance sustainability but also improve comfort for horses. For instance, combining Blueface Leicester wool, Bamboo, and Tussah Silk results in a textile that is durable, breathable, and luxurious, offering superior comfort for horses with sensitive skin (Azam *et al*., 2023). The adoption of these fibres in equine products, particularly for IBH management, reflects the growing demand for sustainable, environmentally friendly, and responsible consumerism in the equestrian industry (Ward, 2023).

Zeolites:
The use of Ag zeolites in horse blanket textiles is a novel approach; there is limited practical research on its application in equine textiles specifically. Zeolites, a natural mineral with exceptional ion exchange capabilities, have shown significant potential in textile applications, particularly when enhanced with antimicrobial agents like silver (Azizi *et al*., 2021). Ag zeolites can also be made synthetically, allowing for greater control over their properties and enabling their tailored use in various textile applications. They have become a powerful tool in textile development, providing antimicrobial protection, moisture management, and enhanced durability (Vasconcelos *et al*., 2023). Integrating Ag zeolites into existing equine textiles, a sustainable solution can be created that combines the natural benefits of fibres with the added advantages of silver's antimicrobial properties. Resulting in a textile that delivers long-term protection against insect bites and bacterial infections (Gong *et al*., 2022; Li *et al*., 2021). Zeolite-based textiles not only enhance the functional properties of the fabric but also contribute to the sustainability of the equine textile industry by reducing the need for frequent replacements and minimising the environmental impact of textile production (Wang *et al*., 2021).

However, there is a lack of lifecycle assessments, so this innovation could represent a promising direction for developing more effective, eco-friendly solutions for IBH management. The growing demand for functional and sustainable textile solutions, especially in the equine sector, highlights a significant advancement in integrating Ag zeolites into textile production. IBH remains a major challenge for equine health, and current treatment options often do not provide long-term protection. Incorporating Ag zeolites into equine textiles, as discussed by Gong *et al*. (2022) and Li *et al*. (2021), offers an innovative method for addressing insect bites and bacterial infections. This approach enhances the antimicrobial properties of the fabric while also improving moisture control and durability, making it a more sustainable and environmentally friendly alternative to traditional treatments. The dual benefits of incorporating Ag zeolites into equine textiles provide increased performance and a reduced environmental impact, positioning this technology as a key development in the future management of IBH.

METHODS

Synthetic Textiles:

All samples were washed at 30°C and air-dried overnight for consistency. Circular pieces (90 mm diameter) of each fabric were tested to evaluate physical properties. Breathability was measured using a Turl dish apparatus, calculating mass loss after 24 hours for water vapour permeability (WVP). Abrasion resistance was tested with a Martindale Abrasion Tester, applying 50,000 rubs and assessing wear at intervals. Burst strength was measured with the Truburst Burst Strength Tester, recording maximum pressure until rupture. Water repellence was evaluated using a spray rating tester, with moisture retention rated from 0 (complete wetting) to 5 (no wetting). Colourfastness was assessed with a Crockmeter, and dye transfer was checked during wet and dry rubbing. Pilling resistance was measured with a Pilling Tester, which visually inspected the pilling on samples rubbed against cork-lined drums. Lastly, tensile strength was tested using a standardised machine for specific fabric samples, following relevant British and international standards to ensure reliability in performance evaluation.

Physical analysis sample 1

1A	1B	1C	1D	1E
				

Figure 2. Sample 1. 1a: Deconstructed using tweezers and a pic to analyse the fabric structure carefully. Sample 1b: Subjected to 50,000 rubs on the Martindale Abrasion Tester to assess durability and resistance to abrasion. Sample 1c: The Truburst burst strength test was evaluated to measure the fabric's resistance to rupture under pressure. Sample 1d: Analysed with the Spray Rating Tester to evaluate the water repellence of the fabric. Sample 1e: Identified as hydrophobic based on its ability to repel water.

Physical analysis sample 2

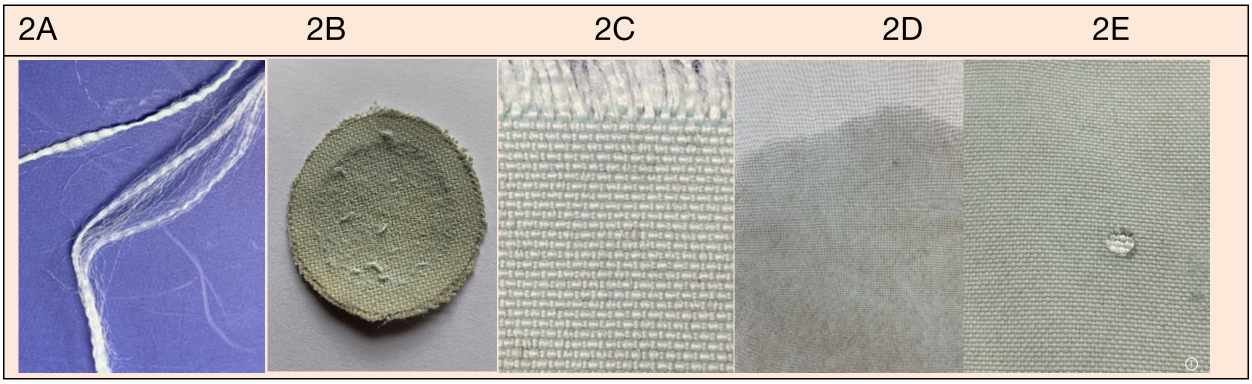

Figure 3. Sample 2. 2a: Deconstructed using tweezers and pulled apart with a pic to analyse the fabric structure, composed of multiple long-strand filament fibres. Coupon 2B: is Subject to 50,000 rubs on the Martindale Abrasion Tester to assess durability and resistance to abrasion. Sample 2c: Evaluated after tensile strength test breaking force of 69.49 psi (converted to N) and tensile strength extension before breaking 17.658kg before tear (converted). Sample 2d was analysed with a Spray Rating Tester to evaluate the water-repellence of the fabric. Sample 2e was identified as hydrophobic based on the ability to repel water. Permethrin

Physical analysis sample 3

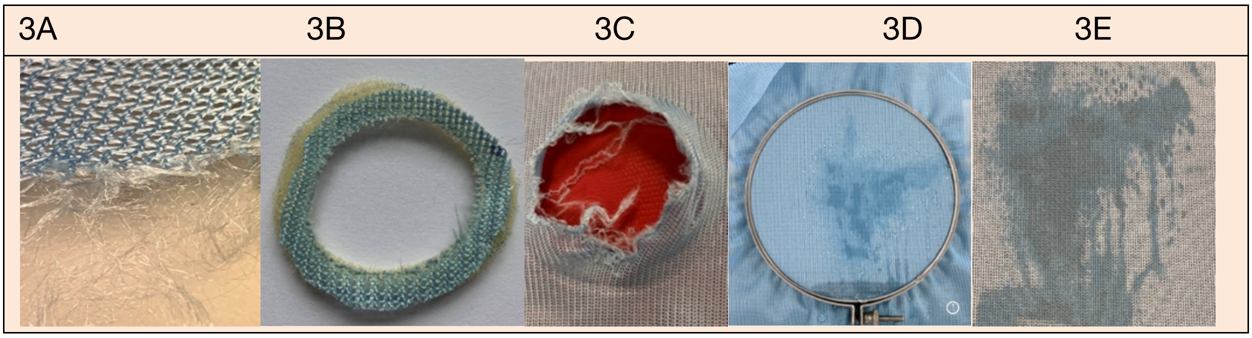

Figure 4. Sample 3. 3a: Deconstructed using tweezers and a pic to analyse the fabric structure. Sample 3b, after 30,000 rubs on the Martindale Abrasion Tester. Sample 3c: The Truburst burst strength test was evaluated to measure the fabric's resistance to rupture under pressure. Sample 3d: Analysed with the Spray Rating Tester, characterised as hydrophilic, confirming its tendency to absorb water rather than repel it. Sample 3e: Composed of filament fibre, highlighting the structural composition of the textile.

Natural Fibre Textile Development

The fibre blend in this study comprises 60% Blueface Leicester Wool (BFLW), 20% Tussah Silk, and 20% Bamboo. The wool was combed to align the fibres and eliminate short strands before blending with tussah silk and bamboo. The tussah silk slivers underwent gilling twice for uniformity. To ensure consistent weight and thickness, the blend was autoleveled. The fibres were drafted using the worsted spinning method, twisted into a single yarn, and plied into twofold yarn. This twofold yarn was twisted in a ‘Z’ direction for added strength. The finishing process included washing to remove impurities and enhance softness, followed by oiling for improved smoothness. The final yarn showcased smoothness, strength, and elasticity, making it suitable for textile applications such as garments and equine products.

Stages of Natural Fibre Development

A B C D E

Figure 5. Natural fibre development: Image A shows processed natural fibres of Bamboo, Tussah Silk, and Blueface Leicester Wool, neatly coiled at the bottom of a container. The fibres appear soft and lustrous, indicative of being well-combed and prepared for blending or spinning processes. Image B: Captures the fibre gilling process. Multiple strands of fibres are aligned and fed into the gilling machine for even distribution and blending, ensuring uniformity before the spinning process. Image C: Displays an autoleveling machine. A fibre strand is fed through the machine, ensuring consistent thickness and weight of the fibre blend before spinning. Image D Shows the spinning setup, with multiple fibre strands running through a spinning frame. The arrangement is organised, with fibres suspended and directed for the worsted spinning process. The tooling-up process here had to be adjusted with the drums pulled away from the spinning frame as the fibres were too soft and slick and kept slipping. Image E Shows the final yarn product. The yarn is soft, consistent, and (Z) twisted, with sections demonstrating washed and oiled samples, showcasing the finishing process for optimal strength and handling.

Experiment 1:

Initial Screening Using Zone of Inhibition

This preliminary experiment followed the BS EN ISO 20743:2021 standard for determining antibacterial activity in textile products. The screening involved preparing bacterial cultures of *S. aureus*, *P. aeruginosa*, *E. coli*, and *A. baumannii*, which were inoculated onto Tryptone Soy Agar (TSA) plates. The textile samples, cut into 1 cm^2 coupons, were cleaned with a Tween and distilled water solution and then incubated overnight. Bacterial cultures were prepared and standardised to an optical density

(OD540) 1.0. Serial dilutions were made, and 100 µl of each bacterial culture was added to the agar plates, onto which three textile samples were placed. Plates were incubated at 37°C for 18 hours and examined for zones of inhibition. A visible cleared zone around the textile indicates antimicrobial efficacy.

Experiment 2:
Preparation of Ag Zeolites and Characterization

Two zeolites were used in the study: synthetic zeolite beta (BEA)[3] and natural clinoptilolite (HEU)[4]. Silver was introduced into these zeolites via ion exchange with AgNO3 (0.01 M). The zeolites were mixed with the silver solution for 48 hours in the dark, centrifuged, and dried. The silver content and structural integrity were analysed using X-ray diffraction (XRD), energy-dispersive X-ray fluorescence (EDXRF), surface area analysis (BET), and scanning electron microscopy (SEM). These characterisations confirmed the successful loading of silver onto the zeolites and provided insights into their surface properties.

Scientific Instruments:

Four instruments were used to characterise the Ag zeolites. Characterisation X-ray diffraction (XRD) analysis of the commercial and Ag zeolites was performed with a PANalytical X'Pert XRD diffractometer employing copper K-α radiation (40 kV and 30 mA) and a PIXcell detector.[5] An energy-dispersive X-ray fluorescence (EDXRF) determined the Ag content using a Rigaku NEX-CG X-ray Fluorescence (XRF) spectrometer.[6] The samples' surface areas were determined from nitrogen adsorption data at 196°C obtained with a Micromeritics ASAP2020 surface area analyser using the Brunauer-Emmett-Teller (BET) equation. Samples were degassed at 200 °C overnight prior to SEM analysis using a Carl Zeiss Supra 40VP Scanning Electron Microscope (SEM).

Experiment 3:
Spot Inoculation Tests for Antimicrobial Efficacy

The antimicrobial activity of Ag zeolites (B-Ag and C-Ag) was assessed through spot inoculation tests on Thioglycolate Agar (TGA) and Lysogeny Broth (LB) agar. Bacterial suspensions of *S. aureus*, *E. coli*, *P. aeruginosa*, and *A. baumannii* were prepared and diluted. Zeolite suspensions at concentrations of 1 mg/ml, 5 mg/ml, and 10 mg/ml

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[3] The code BEA is used to refer to Zeolite Beta, a type of zeolite with a specific crystalline structure that is commonly employed in industrial applications such as catalysis and adsorption.

[4] The code HEU refers to Clinoptilolite, a natural zeolite that is widely used due to its ion-exchange properties and its ability to adsorb various substances.

[5] PANalytical X'Pert XRD diffractometer: Name and model of the machine used for the analysis.

[6] Manufactured by Rigaku Applied Technologies of Ceder Park, Texas, USA. This apparatus is recommended by the manufacturer for measuring ultra-low and trace element concentrations up to high weight percent levels

were prepared, and 25 µl of bacterial culture was added to each zeolite solution. The inoculations were applied to the agar plates in timed intervals (0, 1, 2, 4, 6, 8, 15, and 30 minutes). The plates were incubated at 37°C for 24 hours and visually inspected for bacterial growth. The effectiveness of the Ag zeolites was determined by comparing the bacterial growth on the treated plates to controls.

Experiment 4:
Modification of Synthetic Textiles with Ag Zeolites

The modification process involved treating horse blanket fabric coupons (samples 1-3) with poly(diallyldimethylammonium chloride) (PDDA) to introduce a positive charge on the fabric, facilitating the adsorption of negatively charged Ag zeolites. PDDA was diluted to 0.5 wt% and applied to the fabric, which was then treated with Ag or control zeolites (B-Ag, C-Ag, and their respective controls). The fabric samples were stirred for 1 hour in the zeolite solution, washed, and dried.

Scanning Electron Microscope (SEM) of Zeolite-Modified Textiles

Each sample was prepared by mounting it onto aluminium pin stubs with adhesive carbon tabs and subsequently coated with a thin layer of gold (Au). The samples were then inserted into the SEM chamber for imaging for a magnification of 20,000 times (20 KX). The textile samples treated with Ag zeolites were analysed using a scanning electron microscope (SEM) to evaluate the distribution and bonding of the zeolite particles on the textile fibres. Samples were mounted, gold-coated, and imaged at magnifications ranging from 50x to 25,000x to capture topographical details and verify the uniformity of the zeolite application. The SEM analysis provided visual evidence of Ag zeolite bonding on the textile surface, which is crucial for assessing the durability and effectiveness of the antimicrobial treatment.

RESULTS AND DISCUSSION

Synthetic Textile

The textile selection tests showed varying performance levels among the three samples. Sample 1 had a water vapour permeability (WVP) of 2500 g/m²/24h, offering moderate breathability while maintaining high abrasion resistance with no damage after 50,000 rubs. It showed excellent water repellence (rating of 4) and strong colourfastness, with a pilling resistance score of 5. Sample 2 had the lowest breathability (WVP of 2000 g/m²/24h) due to its dense structure. It maintained good abrasion resistance, overall integrity, and a tensile strength of 17.658 kg before tear. This value was converted to Newtons to maintain consistency with other measurements, highlighting the fabric's moderate resistance to tearing but moderate water resistance (rating of 2). Sample 3 had the highest breathability (WVP of 3000 g/m²/24h) but showed lower durability, with significant wear after 30,000 rubs and no water resistance (rating of 0). It did perform well in colourfastness and pilling resistance, scoring 5. In summary, Sample 1 is the best choice for high-performance applications, Sample 2 is a strong mid-range option, and Sample 3, while affordable, is better suited for lighter use.

Natural Fibre Textile

The yarn from a blend of 60% Bluefaced Leicester Wool (BFLW), 20% Tussah Silk, and 20% Bamboo exhibited several key characteristics. Including BFLW ensured a robust yet soft texture, while Tussah Silk contributed strength, softness, and a luxurious finish, enhancing the yarn's visual appeal. Bamboo added strength to the yarn. The production processes of combing, gilling, and autoleveling resulted in a consistent fibre blend essential for quality spinning. The worsted spinning technique yielded a strong, smooth yarn with minimised slippage from the softer fibres. S-spinning created a smooth texture, and the Z-twist applied during the doubling frame process enhanced strength and cohesion. Post-processing steps, including washing and oiling, refined the yarn further, resulting in a product that was soft, clean, and easy to handle. Ultimately, the yarn proved ideal for fabric production, garment manufacturing, and equine-related products, showcasing excellent performance characteristics while promoting environmental sustainability.

Initial Screening of Selected Textiles

The unmodified textile coupons and agar plates were examined for any potential antimicrobial properties using a zone of inhibition test. For all three samples, no visible zone of inhibition appeared on any of the bacterial inoculated TSA plates at any of the three concentrations. Characteristic colony forming units (CFUs[7]) for each bacteria (judged by colour and appearance) were clearly visible on the textiles in each plate. This study found that the selected textiles showed no inherent antimicrobial functionality. This was a crucial step in demonstrating that any antimicrobial properties of the modified textiles were due to the modification, not the original textiles.

Zeolite Ion Exchange Modification Characterisation

The figures below show the XRD patterns of clinoptilolite (Figure 5) and zeolite beta (Figure 6) before and after the Ag ion exchange. The similarity of the patterns shows that the zeolite structure is preserved in both samples.

[7] Colony forming unit is a measurement of viable bacterial cell numbers able to proliferate and form small colonies.

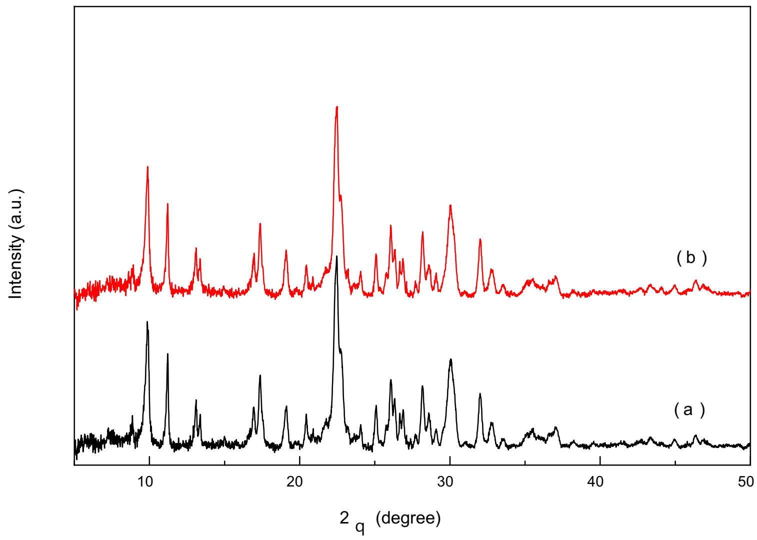

Figure 6. XRD patterns of (a) clinoptilolite zeolite and (b) Ag- clinoptilolite

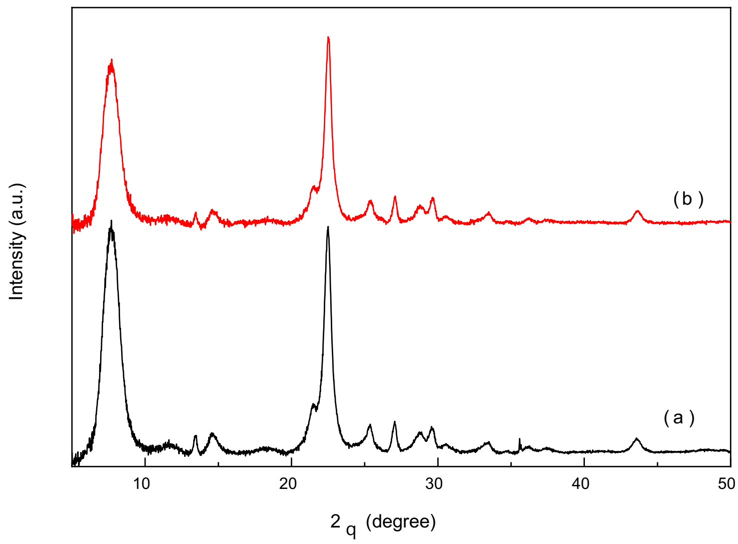

Figure 7. XRD patterns of (a) beta zeolite and (b) Ag-beta.

The XRF data showed a higher silver loading for the clinoptilolite sample compared to zeolite beta (see Table 1). This is due to the zeolite beta's surface area. BET surface areas slightly decreased upon Ag ion exchange, whereas the external surface areas of the Ag zeolite were higher (see Table 1). The ion exchange procedure involved stirring for 48 hours, which may have resulted in slight amorphisation of the samples and reduced particle size, which could explain the differences.

Table 1. Ag content, BET surface areas, and external surface areas of zeolites before and after Ag-modification.

Zeolite	Ag content wt%	SBET (m2 g-1)	SEXT (m2 g-1)
Clinoptilolite	-	25	4
Ag-clinoptilolite	2.5	16	13
Beta	-	504	67
Ag-beta	1.4	483	74

The SEM images show no change in morphology between the zeolites before and after the ion exchange.

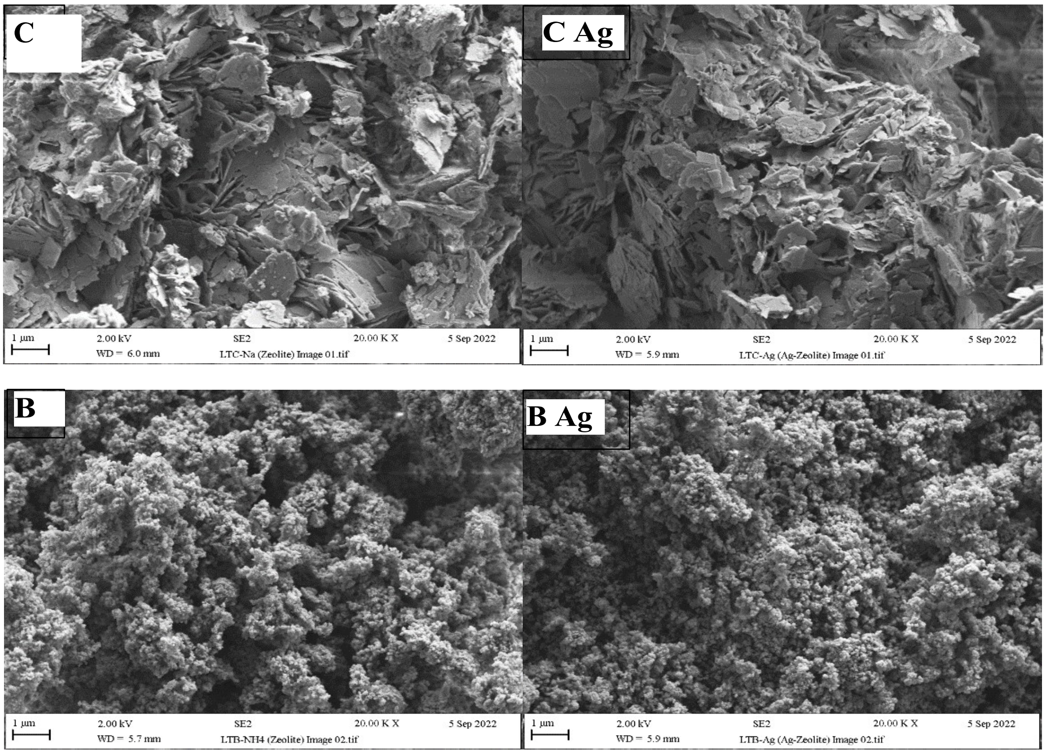

Figure 8. SEM images of the two zeolites (Clinoptilolite above (C control), (C Ag), and beta below(B control), (B Ag), before (left) and after silver exchange (right)

Initial Screening of Selected Textiles

Initial testing showed no antimicrobial activity in the unmodified textile coupons. No visible zones of inhibition appeared on any bacterial-inoculated TSA plates for all three textile samples at any concentration. Bacterial growth (measured by colony-forming units, CFUs) was observed on the textiles, confirming that the textiles did not exhibit inherent antimicrobial properties. This result confirmed that any antimicrobial effect observed in subsequent tests was due to the modification process.

Zeolite Ion Exchange Modification Characterisation

XRD analysis revealed that the silver ion exchange process preserved the zeolite structure, with no significant changes observed in the XRD patterns of clinoptilolite and zeolite beta. The silver content was higher in clinoptilolite (2.5 wt%) compared to zeolite beta (1.4 wt%), as indicated by XRF data. BET surface area analysis showed a slight decrease in surface area after ion exchange, and SEM images showed no significant morphological changes in zeolite crystals before and after modification.

Spot Inoculation Antimicrobial Efficacy

Spot inoculation tests showed that Ag zeolites (B-Ag and C-Ag) exhibited bactericidal effects, with bacterial growth only occurring at the shortest intervals (0-2 minutes). Bacterial growth was absent at longer time intervals, indicating both bacteriostatic and bactericidal properties. The synthetic zeolite B-Ag demonstrated a faster bactericidal effect compared to C-Ag, suggesting higher efficacy in antimicrobial activity.

Fibre–Zeolite Bonding Characterisation

SEM analysis revealed that Ag zeolites were bonded to the textile fibres, with some zeolite particles closely aligned to the fibres and others standing on the surface. High magnification images at 25 KX clearly showed zeolite crystal structures. The bonding of zeolites to the fibres was confirmed, suggesting significant antimicrobial potential, especially as crystal size reduction correlates with increased antimicrobial efficacy.

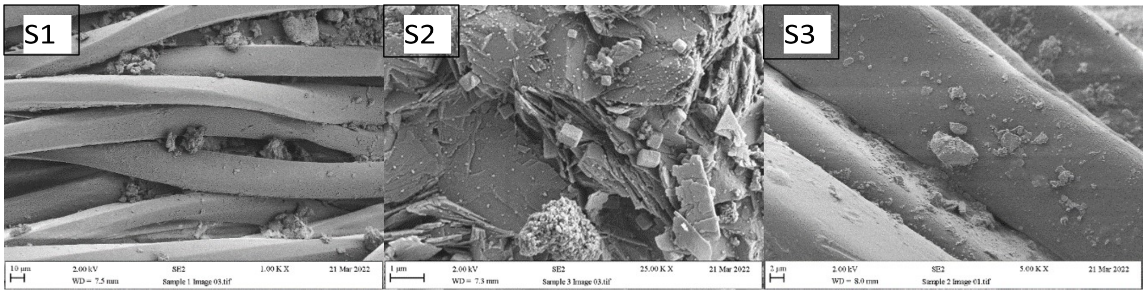

Figure 9. SEM images of Sample 1. with C-Ag at a magnification of 1000x. Sample 2. with B-Ag:
magnification of 5000x. Sample 3. with C-Ag: magnification 1000x.

Antimicrobial Efficacy of Ag Zeolite-Modified Textiles

The Ag zeolites (B-Ag and C-Ag) significantly reduced bacterial growth on modified textile coupons, with most samples showing no bacterial growth after a 24-hour incubation. Control samples (B-NH_4 and C-Na)[8] exhibited substantial bacterial growth, confirming the antibacterial effectiveness of the silver-modified textiles. Exceptions were observed with sample 1 (C-Ag and *E. coli*) and sample 3 (C-Ag and *A. baumannii*)

[8] NH_4 (ammonium) and Na (sodium) refer to the predominant exchangeable cations present in the zeolite's structure. Zeolites are crystalline aluminosilicates with a porous framework that allows them to act as ion exchangers. These cations (NH_4^+ or Na^+) are located within the zeolite's pores or channels and balance the aluminosilicate framework's negative charge. These are used as the controls.

samples, which showed minimal CFUs (1 and 2 counts), indicating strong bactericidal activity despite slight growth.

Long-Term Weathering and Antimicrobial Efficacy

After a 12-month weathering period, Ag-modified textiles (B-Ag and C-Ag) maintained strong bactericidal activity, showing no bacterial growth after incubation. Control samples again displayed substantial bacterial growth. A few exceptions were noted: sample 1 (C-Ag and *P. aeruginosa*) and sample 3 (C-Ag and *P. aeruginosa*) showed minor bacterial growth (2 and 6 CFUs, respectively), with synthetic zeolite Beta exhibiting superior durability and effectiveness over natural zeolite Clinoptilolite. Overall, the Ag-modified textiles retained their antimicrobial properties, demonstrating the durability of the modification over time.

CONCLUSION

This study highlights the promising potential of incorporating Ag zeolites into equine textiles, providing a sustainable and effective solution for managing IBH. By combining the natural benefits of zeolites with the antimicrobial properties of silver, these developed textiles offer long-term management of insect bites and the resulting bacterial infections while also enhancing the overall sustainability of textile production in the equine industry. The successful integration of natural fibres, including Bluefaced Leicester Wool, Tussah Silk, and Bamboo, with advanced textile treatments demonstrates the ability to create textiles that balance performance and environmental responsibility. These findings emphasise the importance of developing multifunctional textiles that cater to the welfare of the equine population and meet the growing demand for eco-friendly solutions in textile manufacturing. This research paves the way for further innovations in antimicrobial textile applications, particularly in veterinary care and other high-performance sectors, promoting both functionality and sustainability.

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