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The Effect of Ammonyx on Some Pathogenic Microbes and Enhance Antimicrobial Activity on Building Worker's Clothes

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Abstract: The most clothing products are in direct contact to human body and they are a very suitable substrate for growth and increase of pathogenic microbes especially bacteria and fungi. In this research, the main aim is to investigate the antimicrobial effectiveness of ammonyx solutions finished on Building Worker's Clothes by immersion method. 60 healthy male subjects (building workers) participated in this study. They were dressed in a cotton/polyester uniform for 14 days and some microbes found on their clothes were investigated. The antimicrobial effect of different ammonyx solutions on the identified microbes was studied by the zone inhabitation method in vitro. In the next step the cotton/polyester uniforms were treated with different solutions of ammonyx like before and the antimicrobial effectiveness was assessed by colony count method at different times and the results were compared with untreated ones. Some mechanical properties of treated cotton/polyester yarn were measured after 30 days and were compared with untreated one. Finally after finishing, scanning electron microscopy (SEM) was used to compare the surfaces of the finished and unfinished specimen. The results show the presence of eight pathogenic microbes on building worker's clothes such as Escherichia coli, Staphylococcus aureus, Aspergillus and Mucor. The inhalation time of treatment on building worker's clothes improved. The amount of colony growth on treated clothes reduced considerably and moreover the mechanical tests results show no significant deterioration effect of properties in comparison to the untreated yarn. The visual examination of the SEM indicated that the antimicrobial treatments were applied usefully on fabrics.

Key words: Pathogenic microbes · Building worker's clothes · Ammonyx · *Escherichia coli* · Fusarium

INTRODUCTION

A wide array of microorganisms lives in a compost pile. Bacteria are especially abundant and are usually divided into several classes based upon the temperatures at which they grow best. The low temperature bacteria are the psychrophiles, which can grow at a temperature as less than -10°C, but whose optimum temperature is 15°C (59°F) or lower. The mesophiles live at medium temperatures, 20-45°C (68-113°F) and include human pathogens. Thermophiles thrive at above 45°C (113°F) and some live at or even above the boiling point of water [1]. Most of the pathogenic microbes on textile goods are the mesophiles class [2].

The growth of microbes on textiles during use and storage negatively affects the wearer as well as the textile itself [2]. The growth of microorganisms on textiles inflicts a range of unwanted effects not only on the textile itself but also on the wearer. These effects include the generation of unpleasant odor, stains and discoloration in the fabric, a reduction in fabric mechanical strength and an increased likelihood of contamination [3, 4]. For these reasons, it is highly desirable that the growth of microbes on textiles be minimized during their use and storage [5]. In order to obtain the greatest benefit, an ideal antimicrobial treatment of textiles should satisfy a number of requirements [1,6]. Firstly, it should be effective against a broad spectrum of bacterial and

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fungal species, but at the same time exhibit low toxicity to consumers, e.g. not cause toxicity, allergy or irritation to the user. Antimicrobial-treated textiles have to meet standards in compatibility tests (cytotoxicity, irritation and sensitization) before marketing. Secondly, the finishing should be durable to laundering, dry cleaning and hot pressing. This is the greatest challenge as textile products are subjected to repeated washing during their life. Thirdly, the finishing should not negatively affect the quality (e.g. physical strength and handle) or appearance of the textile. Finally, the finishing should preferably be compatible with textile chemical processes such as dyeing, be cost effective and not produce harmful substances to the manufacturer and the environment [3,7]. Several major classes of antimicrobial agents are used in the textile industry. They are generally not new as such and have been used in other industries, e.g. as food preservatives, disinfectants, swimming pool sanitizers or in wound dressings. These agents are potent in their bactericidal activity, as indicated by their Minimal Inhibitory Concentration (MIC) values [3, 8, 9].

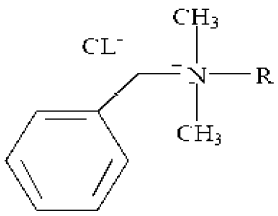
Quaternary ammonium compounds (QACs), particularly those containing chains of 12-18 carbon atoms, have been widely used as disinfectants [10, 17]. These compounds carry a positive charge at the N atom in solution and inflict a variety of detrimental effects on microbes, including damage to cell membranes, denaturation of proteins and disruption of the cell structure [3, 11, 12]. During inactivation of bacterial cells, the quaternary ammonium group remains intact and retains its antimicrobial ability as long as the compound is attached to textiles [3, 13]. Quaternary ammonium halide cationic surfactants are widely used for antibacterial surface-active and detergent properties [1, 14].

Ammonyx shown is one of the conventional quaternary ammonium salts. Its solutions rapidly act as anti-infective agents for a moderately long duration of action. They are active against bacteria some viruses, fungi and protozoa. Solutions are bacteriostatic or bactericidal according to their concentration [5, 15, 16]. The exact mechanism of bacterial action is unknown but it is thought to be due to enzyme inactivation. Activity generally increases with increasing temperature and pH. It has been used in textile industry, as an insecticidal or antimicrobial agent [11, 12, 17]. In this study a conventional antiseptic agent, ammonyx was applied through immersing method for improving clothes's inhibition against some pathogenic microbes and the antibacterial effectiveness of the clothes was evaluated by standard test methods.

MATERIALS AND METHODS

Materials: Ammonyx was purchased from Asalib Co. (Table 1). The clothes were purchased from Kosha Co. Polyester/Cotton blend yarns were prepared from Kosha Co. (20/2 Nm). The pure bacteria were supplied by the Bouali Hospital, Tehran, Iran and all tests were done in the Laboratories of Tarbiat modarres University, Islamic Azad University Science and Research Campus Branch and Islamic Azad University of Shahre-Rey in 2009.

Table 1: Molecular structure and technical data of applied Ammonyx

Trade name	Ammonyx 50
Product	A 50% v/v solution of alkyl benzyl dimethyl ammonium chloride, complying with BP 2003 and USP 26-NK 21 monograph.
 **R: C14H29**	
Appearance at 20°C	Clear liquid
Color	Colorless to pale yellow
Density at 20°C	0.99 $G.cm^{-3}$
Viscosity at 20°C	120 CS
Assay (mmw = 349.8)	50±1
Non-quaternished amine (mmw= 223.3)	0.5 %max
Sulphated ash	0.2 %max
pH (5% in water)	6.5-8.5

Methods: Immersing method was used for adding antibacterial finishes on the test clothes. A polyester/cotton blend fabric was used in this study because it is one of the most frequently used fabrics for scrub suits, lab coats and uniforms [18]. Agar Diffusion Test is used in this research. The agar diffusion tests include AATCC 147-2004 (American Association of Textile Chemists and Colorists), JIS L 1902-2002 (Japanese Industrial Standards) and SN 195920- 1992 (Swiss Norm). They are only qualitative, but are simple to perform and are most suitable when a large number of samples are to be screened for the presence of antimicrobial activity. In these tests, bacterial cells are inoculated on nutrient agar plates over which textile samples are laid for an intimate contact. The plates are then incubated at 37°C for 18-24 h and examined for growth of bacteria directly underneath the fabrics and immediately around the edges of the fabrics (zone of inhibition). No bacterial growth directly underneath the fabric sample indicates the presence of antimicrobial activity. The zone of inhibition should not be expected if the antimicrobial agent is firmly attached to the textile (e.g. covalently) which prevents its diffusion into the agar [19]. If the antimicrobial agent can diffuse into the agar, a zone of inhibition becomes apparent and its size provides some indication of the potency of the antimicrobial activity or the release rate of the active agent. Suspension Test is exemplified by AATCC 100-2004, JIS L 1902-2002 and SN 195924-1992[20-22]. These methods provide quantitative values on the antimicrobial finishing, but are more time-consuming than agar diffusion tests. Typically, a small volume (e.g. 1 ml) of bacterial inoculum in a growth media is fully absorbed into fabric samples of appropriate size without leaving any free liquid. This ensures intimate contact between the fabric and the bacteria. After incubating the inoculated fabrics in sealed jars at 37°C or 27°C for up to 24 h, the bacteria in the fabric are eluted and the total number is determined by serial dilution and plating on nutrient agar plates. Antimicrobial activity, expressed as percentage of reduction, is calculated by comparing the size of the initial population followed by incubation. Appropriate controls, e.g. samples that have gone through the same processing except the antimicrobial finishing, should be included in each experiment to ascertain that the observed decrease in bacterial number is truly due to the antimicrobial finishing. Choosing a calculation equation may be important. It has been observed that two different equations can produce very different results for the same set of data [23-25]. The test fabric, supplied by the Testfabrics Inc. with a code #7409, was 65% Dacron polyester/35% cotton.

60 Male healthy Building Workers (age 21±3 years, stature 185±3 cm and weight 74±10 kg) participated in this study. The subjects were dressed in a cotton/polyester uniform for 14 days. For investigating the kind of bacteria on clothes, (especially pathogenic ones), some fibers were cut from the clothes randomly and immersed in Thioglycolate and Nutrient broth mediums. After incubating for 24 hrs at 37°C, the solutions of each media were sub-cultured in Nutrient and Blood agar mediums and after incubating in Nutrient and Blood agar mediums for 48 h at 37°C the colonies of microbes were cultured by streak test method. For identifying the kind of cultured microbes the gram stain, catalase, oxidase, citrate agar, Christensen's urea broth and TSI agar tests were done. The cultured microbes were kept in skimmed milk in the next step. Ditch plates method was used for evaluating the antibacterial effectiveness of Ammonyx against the detected bacteria on the clothes. Ditch plates were prepared by allowing the Mueller Hinton Agar to solidify in a Petri dish and ditches (with a diameter of approximately 4 mm) were produced on it by removing the agar. Ditches were inoculated by different ammonyx solutions (1/100, 1/500, 1/1000 and 1/2000 v/v solutions of ammonyx). The dishes were incubated for 18 hrs at 37°C to let the ammonyx solutions penetrate into the agar medium. Microbes (stored in skim milk) were mixed with a semi liquid Mueller Hinton Agar (Agar conc. <1%) and added to the inoculated plates. The plates were incubated at 37°C and the zone of inhibition at different time intervals (12, 24, 48, 72, 96, 120, 148, 172, 196, 220, 244 and 268 hrs) were determined. The positive results were repeated three times and the mean of the zone of inhibition was reported for 120hrs. Fibers were sprayed within different solutions of ammonyx (1/100, 1/500, 1/1000, 1 /2000 v/v solutions of ammonyx) and after drying they entered in plates containing the pure microbes and the zone of inhibition was observed until the zone of inhibition disappeared. Every 24 hrs the plates were replaced with new plates of pure microbes. For comparing the antibacterial effectiveness of ammonyx on clothes, the subjects were dressed in a new treated cotton/polyester uniform for 14 days and some microbes found on them were investigated again. First remained untreated and the second was treated with ammonyx solution (1/500 v/v solution of ammonyx by immersing method).

After using clothes used by workers, some fibers cut out of the two samples and the previously described methods were used for culturing and separating the microbes and the antimicrobial effectiveness of ammonyx on clothes was measured by colony count method. Some mechanical properties of untreated and treated (1/500 % v/v solution of ammonyx) polyester/cotton blend yarns were measured by Tensorapid (SDL Co.) after 30 days. The length of every sample was 300 mm and the speed of the test was 999.9 mm/min. An International Standards Instrument ISX-430 SEM was used to compare the surfaces of the finished and unfinished specimen.

RESULTS AND DISCUSSION

This study supports previous works and extends the available information about the quaternary ammonium compound's efficacy and inhabitability against some harmful microbes. Similar to the current study, many researchers found that some quaternary ammonium compounds could inhibit both gram positive and gram negative bacteria. Some researcher investigated the effect of quaternary ammonium compounds treatment on the antibacterial activity of textile fibers and fabrics against various kinds of bacteria. These studies resulted in proving a direct relationship between the degree of antibacterial activity of samples, treated with quaternary ammonium compounds and the amount of their R groups and concentrations. Those authors didn't test ammonyx on clothing fabric such as building worker's clothes and compare with untreated clothes.

Many studies were shown that clothes are carriers of microorganisms such as pathogenic bacteria and odor generating bacteria. In this study the presence of some microbes were proved in the experimental clothes, including Escherichia coli, Staphylococcus aureus, Aspergillus, Candida and Mucor.

The antimicrobial effectiveness of ammonyx solutions on the detected microbes after 120 hrs is listed in (Table 2). According to the results the 1/2000 v/v solution of ammonyx shows no significant antimicrobial effect.

Antibacterial effects of treated fibers with different solutions of ammonyx assessed for the remaining time in the zone of inhibition are listed in (Table 3). Comparison of results of the treated and untreated clothes is listed in (Table 4). It can be seen that the number of colonies growth were decreased about 94% for *Escherichia coli* respectively.

Table 2: Antimicrobial effect of Ammonyx solutions on the Microbes found on clothes

	The zone of inhibition (mm) of Ammonyx solution			
Microbe	1/100 (v/v)	1/500 (v/v)	1/1000 (v/v)	1/ 2000 (v/v)
Escherichia coli	18	15	12	*
Staphylococcus	16	14	*	*
Candida	14	12	*	*
Aspergillus	13	12	*	*
Mucor	13	12	*	*

*: Showed no zone of inhibition

Table 3: Antimicrobial effect of Ammonyx on treated clothes

	Maximum time of inhibition of treated fibers with Ammonyx solution (hr)			
Microbe	1/100 (v/v)	1/500 (v/v)	1/1000 (v/v)	1/ 2000 (v/v)
Escherichia coli	265	165	67	2 8
Staphylococcus	240	95	68	31
Candida	246	76	*	*
Aspergillus	245	99	*	*
Mucor	222	76	*	*

*: Showed no inhibition time

Table 4: The number of colonies growth on untreated and treated clothes with 1/100 Ammonyx (v/v) after 14 days

No. of colonies		
Bacteria	Untreated carpet	Treated carpet
Staphylococcus	9	7.2
Escherichia coli	20	0.9

Table 5: Effect of treating cotton/polyester yarn with Ammonyx on some mechanical properties after 30 days

Mechanical property	Elongation at break (%)	Work of rupture (N.m)	Initial modulus (cN/Tex)	Tenacity (cN/Tex)
Untreated	6.300	6.0000	550.27	37.620
CV	9.344	10.1250	6.69	5.215
Treated	6.103	6.1640	548.50	39.129
CV	9.238	9.2650	3.17	2.283

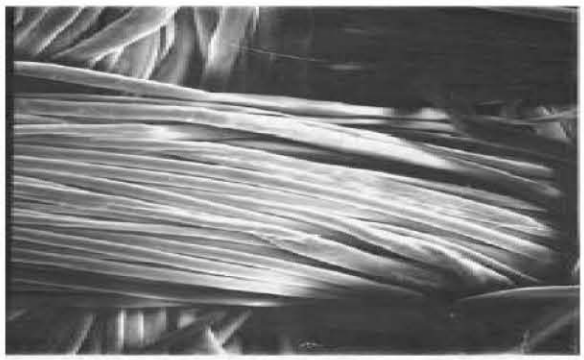


Fig. 1: Scanning electron microscopy of untreated swatch

Fig. 2: Scanning electron microscopy of Ammonyx treated swatch

The effect of 1/100 v/v solution of ammonyx on some mechanical properties of treated clothes in comparison with untreated one is listed in (Table 5). There is no significant deterioration effect on the studied mechanical properties (e.g. the significant level of $\alpha = 0.05$, in Table 5). An International Standards Instruction ISX-430 SEM was used to compare the surface of the finished and unfinished specimens. At 1,000 times magnification, the swatch with no treatment shows an unevenness on the fiber surfaces (Fig. 1). Fiber surfaces with ammonyx treatments looked smooth (Fig. 2). The visual examination of the SEM indicated that the antibacterial treatments were applied successfully to second swatch. The yarns and fabrics surfaces with ammonyx treatments improved handle sensation and comfortable to wear of treated building worker's clothes improved.

CONCLUSIONS

Customer desire for comfort, hygiene and well-being has created a large and rapidly increasing market for antimicrobial textile and clothes. Numerous manufacturers in the textile industry have responded to this demand by launching their brands of antimicrobial products. These products use broad spectrum biocides such as quaternary ammonium compounds as the active agents. The attachment of quaternary ammonium compounds to a textile substrate is believed to be predominantly by ionic interaction between the cationic quaternary ammonium compounds and anionic fiber surface.

The purpose of this study was to examine whether antibacterial finishes can effectively reduce the presence of bacteria on building worker's clothes. Ammonyx was chosen for this study because it is a common antiseptic and it belongs to the group of cationic surface active agents. Considering its charge it can act to link a cationic dye and tend to take up and hold on the surface of natural substrate such as polyester/cotton blend. Also Ammonyx has low toxicity and safe for people and environment.

According to the results the presence of some pathogenic microbes on the clothes such as Escherichia coli and Staphylococcus which can cause many infections was confirmed. So it is worthy to enhance the antimicrobial activity of the clothes with a proper antimicrobial finishing.

The results showed that adding ammonyx as a finish to building worker's clothes can be an effective way to help combat the problem of bacteria contamination on clothes. Our study found that an ammonyx- treated fabric is a good candidate for the fabric in reusable clothes because it fulfills the three requirements for antimicrobial used in textiles reported by thiry (2001): safety, efficacy and ease of production.

Although the kind of microbes on the clothes depends considerably on the environment it was shown that, treating fabric with ammonyx inhibits considerably the growth amount of studied bacteria and in some cases even up to 94%.

Regarding the antibacterial effect of ammonyx against harmful bacteria, the current study found that ammonyx at low concentration could successfully reduce identified bacteria on building worker's clothes. In addition, the process of applying ammonyx through padding and drying is easy and economical and can be easily incorporated into standard processes of a textile mill.

The wash fastness or durability effect against washing of the treated clothes was not given much attention in this study because the interval between washing periods for those textile clothes are not short and during these intervals usually the activity of the antibacterial agent vanishes, as it was seen in the case of ammonyx whose maximum inhibition time with a high concentration (1/100 v/v) was just 265 hrs.

The amount of colony growth on treated clothes reduced considerably and moreover the mechanical tests results show no significant deterioration effect of properties in comparison to the untreated yarn that used in clothes. The visual examination of the SEM indicated that the antimicrobial treatments were applied usefully on fabrics. A successfully finished specimen should look smoother and more uniform compared to the unfinished specimen because the finish improves the surface properties of fibers and yarns. Building worker's clothes surfaces with ammonyx treatments looked smooth and the antibacterial treatments were applied successfully to improve handle sensation of clothes.

As a corollary to this research, an internet search conducted by the researchers revealed no commercially available antibacterial-finished building worker's clothes on the market; therefore, using antibacterial agents to treat building worker's clothes could create a niche product for companies selling building worker's clothes.

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