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Use of Ag-TiO2 and ZnPT Complexes in the Development of Antimicrobial Textiles

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ABSTRACT

In this study, it was aimed to use the synthesized Ag-TiO_2 and commercial ZnPT complexes in the development of antimicrobial textile. Textile materials containing 70% cotton and 30% polyester were used in the study. The complexes were applied on textile material by exhausted process. Antimicrobial activity of textile samples was determined by AATCC 147 method. As a result of these studies, Ag-TiO_2 and ZnPT complexes have antimicrobial activity against all test microorganisms including *E. cloaceae, E. faecalis, S. typhimirium, S. epidermidis, P. vulgaris, Y. pseudotuberculosis, S. aureus, P. aeruginosa, K. pneumoniae, B. subtilis, E. coli, L. monocytogenes* and *C. albicans.* Optimum conditions of application on textile material were determined that application at 50^{o}C for 60 min and drying at 70^{o}C for 60 min. Optimum concentration values for the application were determined as 4g/L for Ag-TiO_2 and 0.01g/L for ZnPT, and these values were shown as bacteriocidal concentration (MBC) by shake-flask method. After the application carried out under optimum conditions, it was observed that the textile samples gained serious antimicrobial properties and formed an inhibition zone larger than 30 mm around the textile materials. Furthermore, it has been determined that the antimicrobial activities of textile materials were durable up to 10 repeated washings and it was permanent for more than 25 days under open air and room temperature conditions. When Ag-TiO_2 and ZnPT are compared, it can be said that ZnPT is more active and more durable. As a result of this study, it has been revealed that Ag-TiO_2 and ZnPT complexes can be used in the development of antimicrobial textiles.

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Ag-TiO_2, application, antimicrobial activity, textile, ZnPT

1. INTRODUCTION

Textile is a material which has an important place in human life since the first ages with its wide product range. In addition to these functions, textile products that are traditionally used for covering, protection and decoration are gaining new features that will meet different needs today [1]. Textile products are environments that provide suitable temperature, humidity, and nutrients for microorganisms to live and multiply in terms of their structures and places of use [2]. The proliferation of microorganisms in textile products causes problems in terms of functional and aesthetic, especially health. In the field of textiles, fungi and bacteria appear as pathogenic microorganisms that cause serious problems. Fungi and bacteria are generally in a symbiotic relationship in textile products [3,4]. Pathogenic microorganisms that can develop in textile products can cause serious infections with negative effects on human health. In particular, surgical site infections have been reported as one of the most important problems of surgery, and it has been reported that the development of surgical site infections may be caused by healthcare workers, patient care equipment, and textile products used in hospitals and operating rooms, often in contact with patient body fluids [5,6].

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Such reasons and especially the epidemics seen in recent years, increasing nosocomial infections and the increase in hygiene awareness have revealed the need for antimicrobial textile products. Many antimicrobial substances have been developed that can be used in the textile industry to meet the increasing need today. These substances have very different working mechanisms from each other according to their chemical structures, working mechanisms, human and environmental effects, adhesion characteristics to the product they are applied, resistance to various external effects, prices, and interactions with microorganisms [7]. It is expected that these materials to be used in the textile industry can protect human health with bacteriostatic or bactericidal effects against the growth of microorganisms, are significantly durable, and have low toxicity for the manufacturer's application and the consumer's wearing [8,9]. Today, antimicrobial agents such as triclosan, chitosan, quaternary ammonium salts, silver, copper, and zinc are used to impart antimicrobial effect to textile products [1,10].

Among these, the antimicrobial property of silver has been evaluated among natural antimicrobial substances known since ancient times. It is reported to have inhibitory activity against approximately 650 bacterial species such as *E. coli, S. aureus, Klesiella* sp. and *Pseudomonas* sp. Although the details of the antimicrobial mechanism of silver have not been clearly explained, it has been reported that silver ions bind to electron-carrying compounds such as thiols, carboxylates, amides and imidazoles found in the structures of enzymes and DNA in microorganisms and inactivate them [11]. The use of silver-containing molecules has a very important place in the development of textile products. Today, it is reported that efficacy against antibiotic-resistant bacteria is achieved by using wound dressings containing different amounts of silver [4]. The fact that silver is skin-friendly and does not cause skin irritation supports the use of silver in the development of textile products. In addition, silver has many advantages such as having a very broad spectrum as an antimicrobial agent, almost no bacterial resistance to silver, non-toxic at low concentrations, and ease of production [12]. Today, silver is used in the production of many commercial antimicrobial synthetic fibers and yarns used in the production of textile products. Examples of these are Ultra-Fresh®, Silpure®, AlphaSan®, Bioactive®, SmartSilver® [13]. Although silver derivatives have a larger market share in different sectors compared to the textile sector, the applications of silver derivatives in the textile industry are increasing rapidly [14]. However, it is seen that different metal nanoantimicrobials such as copper and zinc are used as well as silver is used in the development of antimicrobial textile products [1-10]. In particular, zinc pyrithione with high antimicrobial activity is used in many different products, including textile. Zinc pyrithone (ZnPT) is an organometallic compound with bacteriocidal and fungicidal activity. It is the most used antifungal agent in shampoos for dandruff control. ZnPT was developed in the 1950s and was first used as an anti-dandruff agent in 1961 [15,16,17]. The combination of zinc and pyrithione applied at low concentrations in cell cultures has been found to inhibit the replication of the SARS coronavirus [18]. ZnPT is skin compatible and eco-safe [19]. The main proportion of ZnPT consumption volume is mainly non-textile uses such as the treatment of dandruff, seborrheic dermatitis, and psoriasis [14,20,21]. Other applications include textiles, adhesives, paints, wire/cable insulation and floor coatings [21]. While the use of zinc pyrithone for creams, sprays and shampoos is at serious levels, its use in textiles is considerably lower than textile antimicrobials such as Si-QAC (silane-quaternary ammonium compounds) and TCS (triclosan) [14]. In the face of increasing demand, its applications in the textile industry are increasing rapidly.

In this study, it was aimed to use the synthesized Ag-TiO_2 and commercial ZnPT complexes in the development of antimicrobial textile, which inhibit the growth of microorganisms or completely eliminate them. Antimicrobial textile is required in many different sectors such as health care, apparel, households [22]. There has been very little study on the use of Ag-TiO_2 and ZnPT in the development of antimicrobial textile and there are no detailed studies such as fabric application, antimicrobial activity of the fabric after application. So, in this study, the use of these complexes in the development of antimicrobial textile products was examined in detail. Firstly, textile application of synthesized Ag-TiO_2 and commercially ZnPT complexes was optimized, before and after the application of the complexes; antimicrobial activity was determined against 13 different microorganisms, including 12 bacteria [7 Gram (-) and 5 Gram (+)] and 1 yeast, it was determined that minimal inhibition concentrations (MIC), minimum bactericidal concentrations (MBC) and optimum concentration values for application.

2. MATERIAL AND METHOD

2.1 Material

2.1.1. Chemicals and Textile samples

Ag-TiO_2 (Silver-Titanium Dioxide) complex was synthesized by modified procedure [23]. The Zinc Pyrithione (ZnPT), Trisodium Citrate (TSS), Sodium Ductosate ($C_{20}H_{37}NaO_7S$), Silver Nitrate ($AgNO_3$), Nitric acid (HNO_3), Acetic Acid (CH_3COOH), Magnesium Chloride ($MgCl_2$), Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB) were procured from Merck. Binder Pac and Wetting Agent were obtained from CHT Chemical. Textile material containing 70% cotton and 30% polyester was used in the study. The textile material is a printed knit fabric with wight of 180 g/m^2. The prepared textile samples were cut in 2.5x2.5cm² (160mg) for use in multiple repetitions such as washing tests. Furthermore, the other textile samples were prepared as 2.5x3.5cm² (238mg) for use in non-repetitive studies such as application tests.

2.1.2. Microorganisms

Test microorganisms used in antimicrobial activity analysis; *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Salmonella typhimirium* ATCC 14028, *Proteus vulgaris* ATCC 13315, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomanas aeruginosa* ATCC 27853, *Enterobacter cloaceae* ATCC 13047, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 13883, *Listeria monocytogenes* ATCC 7644, *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 90028 were obtained from Düzce University, Faculty of Arts and Sciences, Biology Department, Molecular Biology Department. Test bacteria were grown using MHA and MHB media. Yeast was grown at 37°C for 16-18h using PDA and PDB media.

2.2 Method

2.2.1. Synthesis of Ag-TiO_2

Firstly, a solution of TSS (0.7mmol) and $AgNO_3$ (0.32mmol) was prepared in distilled H_2O and mixed at 80°C. And then TiO_2 (0.06mol) was added to the solution and mixing was continued. The resulting solution was cooled to 50°C and stirred for 24h. Then it was adjusted to pH 1-2 with HNO_3 and kept at 50°C for 24h and then dried at 105°C for 24h. In addition, two different forms of Ag-TiO_2 complex, calcined and non-calcined were used. Calcination was performed at 600°C for 2h and was used to increase the activity. The synthesized nanoparticles were characterized by FTIR spectroscopy (Shimadzu, IRPrestige 21) and silver nanoparticles were visualized by SEM (FEI-Quanta FEG 250).

2.2.2. Determination of Antimicrobial Activities of Ag-TiO_2 and ZnPT Complexes

In this study, the antimicrobial activities of the complexes were tested both directly in solid form and in solution form. The solution of Ag-TiO_2 complex was prepared by dissolving Ag-TiO_2 (7%) and $C_{20}H_{37}NaO_7S$ (7%) in 100 ml of distilled H_2O. The solution of ZnPT complex was prepared by dissolving ZnPT (50%) in distilled H_2O. Then complexes were diluted. Antimicrobial activity test directly in solid form and solution form; was carried out by agar well diffusion and agar disc diffusion method. In all methods, test microorganisms were inoculated into MHB/PDB medium at 37^{o}C for 16-18h in a shaking incubator. At the end of the incubation period, the 600nm absorbance of the microorganism cultures was measured in the spectrophotometer (Mapada) and the cultures were diluted with sterile distilled H_2O to be approximately 1×10^7-1×10^8 CFU/ml. 100µl of diluted samples were taken and spread on petri dishes containing MHA and PDA. *For direct antimicrobial activity test*, Ag-TiO_2 in solid form was placed on these petri dishes. *For Agar Well Diffusion Test*; 6-8mm diameter wells were created in these petri dishes and 100µl of Ag-TiO_2 (4g/l) and ZnPT (4g/l) complexes obtained in dissolved form were added to each well. *For Agar Disk Diffusion Test*; sterile discs were placed on the agar surface with the help of a forceps and 10µl of Ag-TiO_2 and ZnPT complexes were absorbed into the discs. Antibiotic (Ciprofloxacin, CIP.30mg; Bioanalyse) was used as a positive control and studies were performed in duplicate. The samples obtained from three different tests were incubated at 37°C for 16-18h and the zone diameters formed around the substances, wells and discs were measured and recorded at the end of the incubation period [24].

2.2.3. Application of Ag-TiO_2 and ZnPT Complex on to the Textile Surface

The exhausted process was performed on the prepared textile samples using solutions prepared from Ag-TiO_2 and ZnPT complexes. Before the Ag-TiO_2 complex was applied, its pH was adjusted to 4.5-5.0 using citric acid to increase adhesion on the textile samples. While performing the application process with ZnPT complex, it is used in today's textile industry to increase the adhesion; Binder Pac: 10g/l, Wetting Agent: 1g/l, Acetic Acid: 1g/l and Magnesium Chloride: 1g/l were used. The parameters analyzed to determine the optimum application conditions are shown in Table 1. These conditions were applied at different times for both Ag-TiO_2 complex and ZnPT complex. Antimicrobial activity of textile samples after application was determined by AATCC 147 method. Firstly, the textile samples obtained after the different applications were placed in the petri dishes prepared as described in 2.2.2. Untreated textile samples were used as negative control. Samples were incubated for 16-18h at 37°C [24,25].

2.2.4. Determination of Minimum Inhibitory Concentration (MIC)

The textile samples were treated with different concentrations of Ag-TiO_2 (0.1 g/l, 0.5 g/l, 1 g/l, 2 g/l, and 4 g/l) and of ZnPT (0.01 g/l, 0.1 g/l, 0.5 g/l, 1 g/l, and 2 g/l) at 50°C for 60 min (Table 2). These concentrations were applied at different times for both Ag-TiO_2 complex and ZnPT complex. The samples were removed at the end of the process and were dried in a drying oven at 70°C for 60 min. In this study, optimum conditions determined in the previous study were used for the application and drying processes. The antimicrobial activity test was performed

[24,25].

Table 1. The parameters used to determine the optimum application

Antimicrobial Complex	Concentration	Application Time	Application Temperature	Drying Process	Indicator Microorganisms
ZnPT /Ag-TiO_2	2 g/l - 4 g/l	30 min	25ºC 50ºC 70ºC 90 ºC	70 ºC for 120 min / 70 ºC for 60 min	*B. subtilis* *E. coli* *L. monocytogenes* *P. vulgaris*
		60 min	25ºC 50 ºC 70ºC 90ºC		*B. subtilis* *Y. pseudotuberculosis*
		120 min	25ºC 50ºC 70ºC 90ºC		*B. subtilis* *E. coli* *L. monocytogenes* *P. vulgaris*

Table 2. The parameters of minimum inhibitory concentration

Antimicrobial Complex	Concentration	Application Time	Application Temperature	Drying Process	Indicator Microorganisms
ZnPT / Ag-TiO_2	0.01 g/l 0.1 g/l 0.5 g/l 1 g/l 2 g/l 4 g/l	60 min	50ºC	70 ºC for 60 min	*E. faecalis* *S. aureus* *S. epidermidis* *S. typhimirium* *P. vulgaris* *Y. pseudotuberculosis* *P. aeruginosa* *E. cloaceaceae* *E. coli* *K. pneumoniae* *L. monocytogenes* *B. subtilis* *C. albicans*

2.2.5. Determination of Minimum Bactericidal Concentration (MBC)

Shake-flask method (ASTM E2149-01) was used for MBC test. Applied textile samples were placed in liquid medium (MHB) containing *B. subtilis* ($2x10^5$ CFU/ml) preferred as the test pathogen, and incubated at 240 rpm and 37°C. After the incubation 0th, 1st, and 24th, 100µl of sample was taken and spread on the MHA surface and bacterial counts were calculated with the following formula [26,27].

% Decrease= [A - B / A] x 100 (A=Initial CFU/ml, B=CFU/ml after incubation)

2.2.6. Stability Tests

Firstly, textile samples were applied with Ag-TiO_2 and ZnPT complexes under optimum conditions to see the antibacterial activity persistence and time-dependent variation of the textile samples. Then, the textile samples were washed 10 times with distilled H_2O at 35ºC for 15min and dried at 70ºC for 60min after each wash. The antimicrobial activities of the samples obtained after each wash were tested [24]. In addition, for the stability test, the textile samples that were not washed after the application were kept under open air and room temperature conditions for 1 day, 3 days and 25 days, and then, the antimicrobial activities of the textile samples obtained at the end of these periods were evaluated [24,25].

2.2.7. Combination of Complexes

In this study, optimum concentrations of complexes determined in the previous study were used. Ag-TiO_2 (4 g/l) and ZnPT (0.01 g/l) were mixed , and textile samples were applied with this combination of complexes at 50°C in 60min and then drying at 70°C in 60min. Antimicrobial activity of textile samples after application was determined by AATCC 147 method [24,25].

3. RESULTS AND DISCUSSION

3.1. Ag-TiO_2 and ZnPT Complexes

In this study, synthesized Ag-TiO_2 and commercial ZnPT complexes were used to obtain antimicrobial textile products. In a previous study, it was stated that composite materials designed using TiO_2 nanoparticles have high antimicrobial activity and that TiO_2 nanoparticles have self-sterilizing properties [28]. In another study, homogeneously polypropylene and silver-containing nanocomposites were produced and characterized by electron microscopy, and it was observed that these nanocomposites have antibacterial activity even at very low concentrations [29]. In this study, considering the molecules with proven antimicrobial properties in this way, Ag-TiO_2 complex was synthesized and characterized by electron microscopy and FTIR spectroscopy to be used in antimicrobial textile production (Figure 1 and 2). The commercial ZnPT complex, on the other hand, is known by many studies to have high antimicrobial activity [14,30,31,32]. However, there are very few studies in the literature on its use to develop antimicrobial textile products [19,33].

3.2. Antimicrobial Activities of Ag-TiO_2 and ZnPT Complexes

It was determined that Ag-TiO_2 complexes (with/without calcination) in solid form showed antimicrobial activity against all microorganisms (Figure 3, Column 1 and Table 3). In dissolved form; Ag-TiO_2 complexes (with/without calcination) are antimicrobial against Gram (-) bacteria as *E. cloaceae, S. typhimirium, P. vulgaris, P. aeruginosa, K. pneumoniae, E. coli* except for *Y. pseudotuberclusis* and *C. albicans* was found to lose its activity. It was found to have inhibitory activity against *E. faecalis, S. epidermidis, Y. pseudotuberculosis, S. aureus, B. subtilis*, and *L. monocytogenes* bacteria. As the cause of the loss of antimicrobial activity; it can be said that Ag-TiO_2 is not completely dissolved in 100 ml distilled H_2O when mixed in 1:1 ratio of $C_{20}H_{37}NaO_7S$ and accordingly the amount of Ag-TiO_2 is insufficient for antimicrobial activity. However, it was determined that the ZnPT complex had high inhibitory activity against all test microorganisms (Figure 3, Column 2-3 and Table 3).

3.3. Optimum Application Parameters of Ag-TiO_2 and ZnPT Complexes

In this study, the exhausted process was preferred due to its widespread use in the textile industry and the easy applicability of the chemicals used to fiber and textile products. The antimicrobial activity of textile samples applied under different conditions was investigated. As a result of the research, optimum application conditions of Ag-TiO_2 and ZnPT complexes; application at 50°C in 60min and then drying at 70°C in 60min. The inhibition of the microorganisms was only present at the point of contact with the agar at different application conditions as 25°C, 70°C and 90°C but not around the textile sample. So, the best condition for application was accepted at 50°C in 60min since it creates a zone larger than 30 mm around the textile samples. Furthermore, it was observed that different drying or application times did not cause serious changes in the inhibition activity. Therefore, easily applicable times were preferred. Textile samples obtained under optimum conditions were found to have high antimicrobial activity against 13 microorganisms (*Y. pseudotuberclusis, E. cloaceae, S. typhimirium, P. vulgaris, P. aeruginosa, K. pneumoniae, E. coli, E. faecalis, S. epidermidis, S. aureus, B. subtilis, L. monocytogenes* and *C. albicans*) (Figure 4).

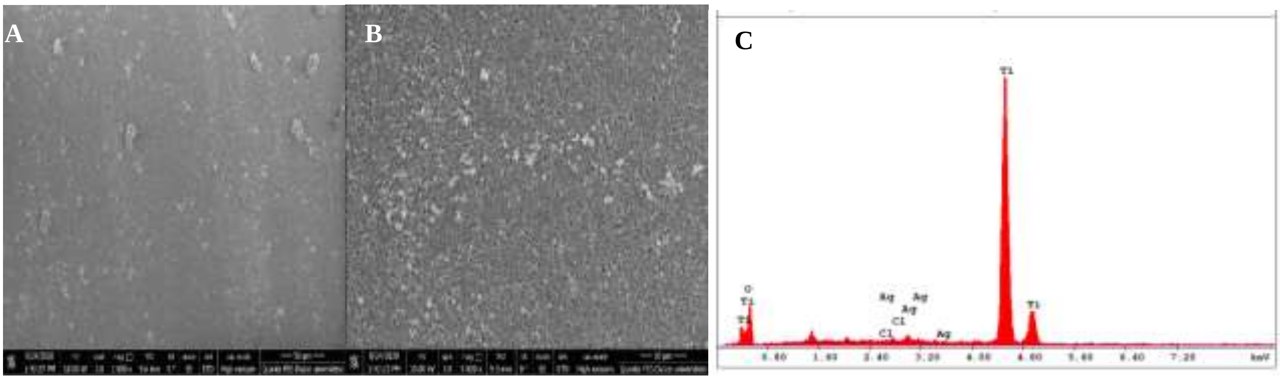

Figure 1. SEM images of Ag-TiO_2 at different magnifications and EDX analysis. [A]; 1000x [B]; 5000x [C]; EDX Spectra

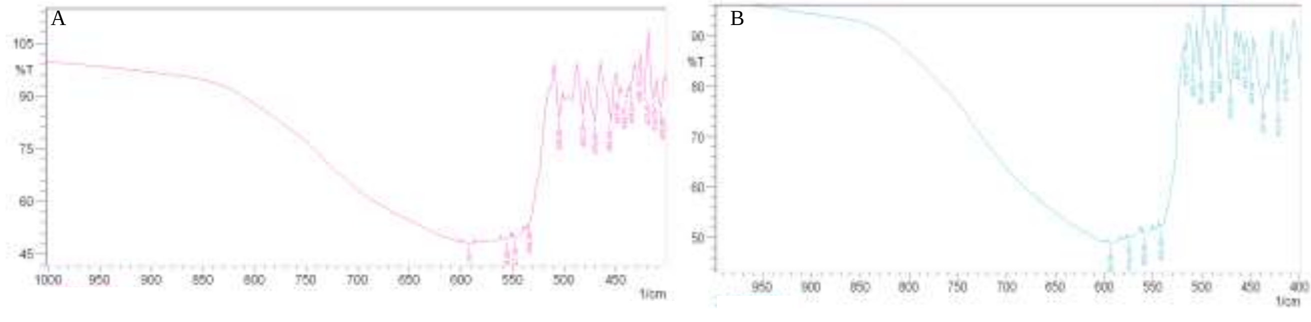

Figure 2. FTIR results of two forms of Ag-TiO_2. [A]; Ag-TiO_2 (Calcinated), [B]; Ag-TiO_2 (Non-Calcination).

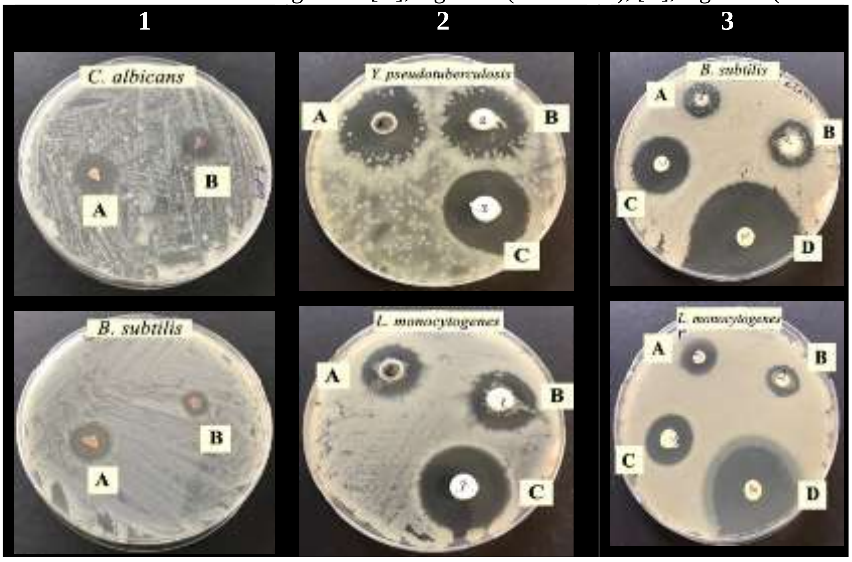

Figure 3. Antimicrobial activity test results of Ag-TiO_2. Column 1. Direct activity test. [A]; Ag-TiO_2 (Calcinated), [B]; Ag-TiO_2 (No Calcination). Column 2.Agar well diffusion test. [A]; Ag-TiO_2 (Calcinated), [B]; Ag-TiO_2 (No Calcination), [C]; ZnPT. Column 3.Agar disc diffusion test. [A]; Ag-TiO_2 (Calcinated), [B]; Ag-TiO_2 (No Calcination), [C]; ZnPT, [D]; Control.

Table 3. Results of antimicrobial activity test of Ag-TiO_2 and ZnPT complexes

Microorganisms	Zone of Inhibition (mm) ZnPT		Ag-TiO_2 (Calcinated)		Ag-TiO_2 (No Calcination)		C
	Well Diffusion Test	Disc Diffusion Test	Well Diffusion Test	Disc Diffusion Test	Well Diffusion Test	Disc Diffusion Test	
E. cloaceae	29	24	-	-	-	-	40
E. faecalis	28	23	16	18	16	18	30
S. typhimirium	28	20	-	-	-	-	41
S. epidermidis	33	22	16	16	16	16	33
P. vulgaris	29	24	-	-	-	-	47
Y. pseudotuberculosis	27	32	31	21	31	23	40
S. aureus	32	31	16	20	16	20	33
P. aeruginosa	27	22	-	-	-	-	43
K. pneumoniae	40	33	-	-	-	-	41
B. subtilis	30	24	23	19	22	20	40
E. coli	27	21	-	-	-	-	38
L. monocytogenes	30	19	19	16	21	16	33
C. albicans	28	28	-	-	-	-	-

C; Positive Control (Ciprofloxacin; 30 mg)

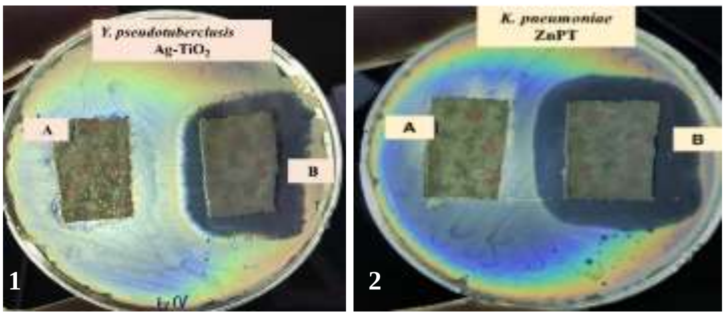

Figure 4. Optimum application conditions.; 1: [A]; Control, [B]; Ag-TiO_2 (4g/l). 2: [A]; Control, [B]; ZnPT (0.01g/l)

It was observed that Ag-TiO_2 complex, which was observed to lose its activity against some microorganisms with antimicrobial activity tests performed after the dissolution process in well diffusion test, regained activity against 7 microorganisms as (*E. cloaceae, S. typhimirium, P. vulgaris, P. aeruginosa, K. pneumoniae, E. coli* and *C. albicans*) after textile application with crosslinker (citric acid). This situation can be explained by the increase in the concentration of the substance applied to the textile material thanks to citric acid. Furthermore, the solid Ag-TiO_2 have already determined that inhibition activity have against all microorganisms with disc diffusion test, so it is an expected result. In previous studies, it was determined that a certain concentration should be reached to increase the antimicrobial properties of chemicals [34]. In this study, it was observed that the antimicrobial activity of the Ag-TiO_2 complex increased depending on the concentration increase. While approximately 400µg of Ag-TiO_2 was used in the well diffusion test, 6 mg of Ag-TiO_2 was applied (the amount of substance absorbed by the fabric) in the application experiments. Additionally, this situation can be explained by the increase of the concentration as well as the attachment of the water-insoluble groups to the textile samples with binders.

In the studies, it is known that silver-containing metal ions are one of the most used materials in the production of antimicrobial fiber [34], and in this study, a broad spectrum and high antimicrobial activity textile product was obtained by the application of Ag-TiO_2 to textile samples. In another study conducted in previous years, silver nanoparticles were prepared by filtering from fungal biomass. Silver nanoparticles were applied to textile materials with/without crosslinker. It was determined that these textile materials inhibited *S. aureus* (97%) and *E. coli* (91%) [35]. In another study, silver nanoparticle solution was applied to cotton textile samples with PVOH (polyvinyl alcohol) crosslinker. The application process was carried out at 140, 150 and 160ºC in 1, 2 and 3 min. It was observed that the textile samples after application had high antimicrobial activity against *S. aureus* and *E. coli* [36]. In another study, it was aimed to develop antimicrobial cotton textile surfaces using zinc oxide nanoparticles. The synthesized zinc oxide nanoparticles were applied to cotton textile materials using impregnation and drying methods. It has been observed that the obtained materials have very high antibacterial activity against *S. aureus* [37]. Compared to other studies, in this study, silver and zinc-containing complexes could be applied to cotton textile samples for a longer time (60 min) at lower temperatures (50°C), and that the textile samples obtained result of the application were highly antimicrobial not only against *S. aureus* and *E. coli*, but also against 13 different microorganisms including fungi.

In this study, while determining the application parameters, antimicrobial activity properties of textile samples were increased by using cross-linking chemicals such as citric acid for Ag-TiO_2, Binder Pac, WLA, Acetic Acid and Magnesium Chloride for ZnPT. The use of such crosslinking chemicals is quite common in the production of antimicrobial textile products. Similarly to this study, in a study, it is seen that citric acid is used to ensure the cross-linking of chitosan to the textile material [38]. In another study in the literature, a nano-sized material from sericin and TiO_2 was developed to impart antimicrobial property to the textile sample and this material was applied the textile samples by using polysaccharide acid as a crosslinker and alone. Antibacterial activity of the applied cotton textile samples against *S. aureus* and *E. coli* was determined. A significant contribution of the use of cross-linking chemicals to antimicrobial activity has been revealed [39].

3.4. Optimum concentration and MIC

The MIC value of Ag-TiO_2 complex for *C. albicans* was determined as 4g/l, *E. cloaceae,* and *S. typhimirium* was determined as 1g/l and for *Y. pseudotuberclusis*, *P. vulgaris, P. aeruginosa, K. pneumoniae, E. coli, E. faecalis, S. epidermidis S. aureus*, and *L. monocytogenes* was determined as 0.5g/l, and *B. subtilis* was determined as 0.1g/l, and MIC value of ZnPT complex for all test microorganisms was determined as 0.005g/l (Table 4).

The optimum concentration of Ag-TiO_2 complex was determined as 4g/l due to reasons such as having high antimicrobial activity, being more stable, and being more durable. In another study, silver nanoparticle solution was prepared at concentrations of 5, 10, 15 and 20g/l and after the application process using PVOH (polyvinyl alcohol) as a crosslinker, textile samples were obtained at these concentrations without serious differences showed high antimicrobial activity against bacteria as *S. aureus* and *E. coli* [36]. The optimum concentration of ZnPT complex was determined as 0.01g/l (=MIC). In a study conducted in 2017, it was determined that the cotton textile sample obtained by the application of 12g/l ZnPT complex had a bacteriostatic effect against *E. coli* [19]. It is seen that a concentration approximately 3 times higher than the optimum ZnPT value found in this study is used. Similar binders were used in this study, and the different application conditions suggest that this is the result.

3.5. Minimum Bactericidal Concentration (MBC)

It was observed that Ag-TiO_2 (4g/l) complex reduced the number of *B. subtilis* (78.46%) within 1h, and bacterial growth was completely eliminated within 24h, and it can be said that 4g/l of Ag-TiO_2 has bactericidal activity (Figure 5). It was determined that the ZnPT (0.01g/l) complex showed its effect rapidly even within 1h and had strong bactericidal activity (Table 5). However, in a study conducted in 2017, it was determined that the ZnPT (12g/l) complex had a bacteriostatic effect against *E. coli* and *S.*

aureus [19].

3.6. Stability Results

Washing resistance can vary depending on the infinite property of the active substance used. As a result of washing tests, it was observed that the antibacterial activity of the complexes continued until the 10th wash (Figure 6). However, it is seen that the Ag-TiO_2 (4g/l application) complex has lost its effectiveness significantly (<70%) as a result of washings, and a loss of activity (<50%) is observed in the stability tests performed in the open air. It was observed that the ZnPT (0.01g/l application) complex was much more resistant to washing tests, and there was no loss of activity even after 10 washings. The time-dependent change was determined to be still active on the 25th day. However, it is seen that there is a serious loss of activity (<50%) in the stability tests performed under open air and room temperature conditions (Figure 7).

Table 4. Antimicrobial efficiency test results of Ag-TiO_2 and ZnPT complexes

Microorganisms	**Ag-TiO₂ (g/l)**					**ZnPT (g/l)**						
	0.1	**0.5**	**1**	**2**	**4**	**0.001**	**0.005**	**0.01**	**0.1**	**0.5**	**1**	**2**
E. cloaceae	-	-	31	33	34	-	+	33	34	35	35	38
E. faecalis	-	+	32	35	37	-	+	34	35	37	40	39
S. typhimirium	-	-	33	34	35	-	+	33	35	36	38	40
S. epidermidis	-	+	32	34	35	-	+	32	33	36	36	37
P. vulgaris	-	+	33	35	37	-	+	31	32	34	35	39
Y. pseudotuberculosis	-	+	34	41	42	-	+	31	32	33	34	33
S. aureus	-	+	32	34	36	-	+	33	35	33	35	39
P. aeruginosa	-	+	32	34	37	-	+	31	34	34	37	40
K. pneumoniae	-	+	32	35	35	-	+	31	43	36	36	43
B. subtilis	+	32	33	37	40	-	+	34	39	40	43	45
E. coli	-	+	32	33	35	-	+	33	35	36	38	40
L. monocytogenes	-	+	32	35	36	-	+	31	33	36	35	40
C. albicans	-	-	-	-	+	-	+	35	40	43	44	50

(+); There is inhibition of the microorganism at the point of contact with the agar, but not around the textile sample. (-); The inhibition of the microorganism is not present at any point in the textile sample.

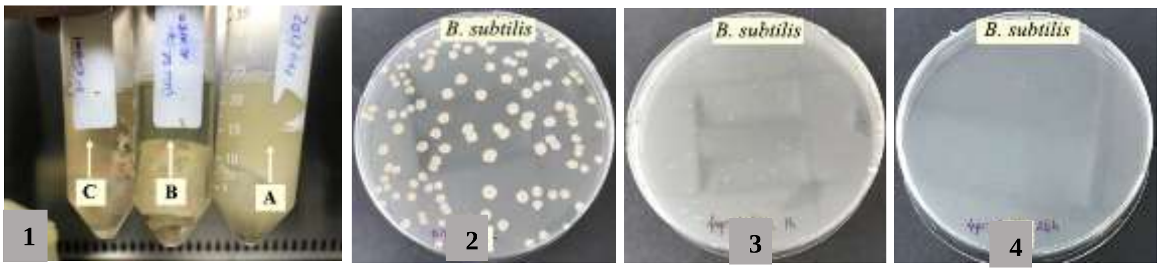

Figure 5. MBC by the shake-flask method. **1.** Incubation of textile samples applied with Ag-TiO_2 (4g/l) and ZnPT (0.01g/l) complexes with *B. subtilis* ($2x10^5$ CFU/ml) **2.** 0th hour of incubation CFU/ml **3.** 1st hour of incubation CFU/ml **4.** 24th hour of incubation CFU/ml.

Table 5. Shake-flask method

Textile samples	**CFU / 1 ml**			**% Decreased vitality (1h)**	**% Decreased vitality (24h)**
	0 h	**1 h**	**24 h**		
Application of Ag-TiO_2 (4g/l)	1300	280	0	78.46 %	100 %
Application of ZnPT (0,01g/l)	90	0	0	100 %	100 %
No application	>300	>300	>300	0	0

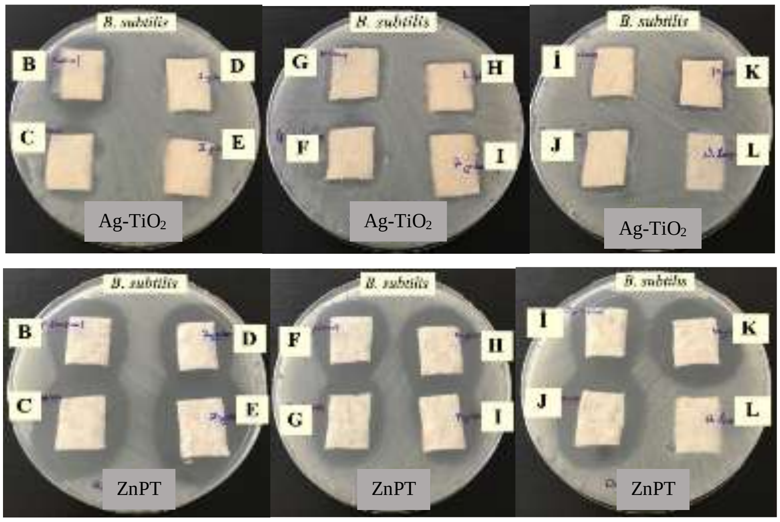

Figure 6. Washing test results. [B]; Positive Control, [C]; 1st wash, [D]; 2nd wash, [E]; 3rd wash, [F]; 4th wash, [G]; 5th wash, [H]; 6th wash, [I]; 7th wash, [İ]; 9th wash, [J]; 8th wash, [K]; 10th wash, [L]; Negative Control.

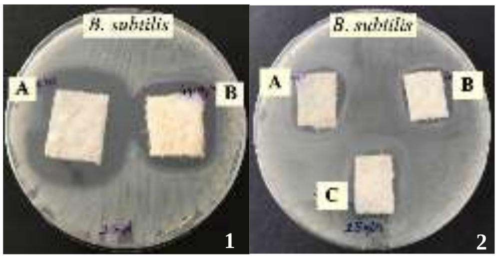

Figure 7. The time-dependent change results. **1.** 3rd day antibacterial activity results. [A]; 0.01g/l ZnPT, [B]; 4g/l Ag-TiO_2 **2.** 25th day antibacterial activity results. [A]; ZnPT (0.01g/l) [B]; Ag-TiO_2 (4g/l) [C]; Ag-TiO_2/ZnPT (4g/l-0.01g/l)

In a study, silver nanoparticles were applied to textile materials with and without crosslinker, followed by 20 washings, and it was determined that the antibacterial activity was significantly permanent. It has been proven that the addition of crosslinking chemicals to the finishing process has a positive effect on the permanence [35]. In a different study, cotton textile with antimicrobial properties was obtained by using zinc oxide nanoparticles, and then it was determined that the obtained materials had high antibacterial activity against *S. aureus* and showed resistance up to 25 washings [37]. In another study, Ag-NP material was applied to the surface of textile samples, and it was determined that even after 20 washing, the substance showed over 95% antibacterial activity against *S. aureus* and *E. coli* [40]. In this study, it was determined that the two complexes showed resistance up to 10 washings. While determining the application parameters, antimicrobial activity gain properties, stability and washing resistance of cotton fabric were increased by using cross-linking chemicals (citric acid for Ag-TiO_2, Binder Pac, WLA, Acetic Acid and Magnesium Chloride for ZnPT). However, as stated in the studies, the use of cross-linking chemicals does not have a significant effect after 20-40 washings [39].

3.7. Combination of Complexes

As a result of the study, it was determined that the antimicrobial activity of the textile samples obtained by the application of the mixtures of Ag-TiO_2 (4g/l) and ZnPT (0.01g/l) complexes decreased against all test microorganisms (Figure 8). An antagonistic interaction was observed between the two complexes. This situation can be explained as that the complexes interact with each other and then their activity declines.

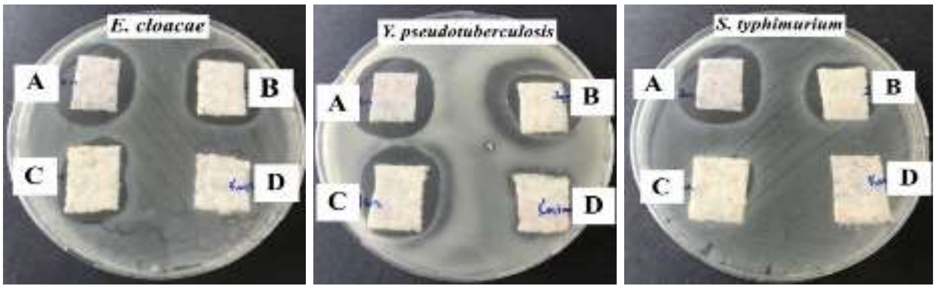

Figure 8. Effects of combination. [A]; ZnPT-0.01g/l, [B]; Ag-TiO_2-4g/l, [C]; Ag-TiO_2 (4g/l)/ZnPT (0.01g/l), [D]; Negative Control.

4. CONCLUSION

Textile products are very suitable environments for microorganisms to live and grow [2]. The epidemics in recent years and the increase in hygiene awareness have led to an increase in the need for antimicrobial textile products. Recently, the production of bacteriostatic fibers and yarns in antimicrobial textile production has been increasing rapidly, and most of these fibers contain antimicrobial chemicals based on silver, copper, and zinc metal complexes [41]. In this study, it was aimed to investigate the possibilities of using the synthesized molecule (Ag-TiO_2) and the commercial molecule (ZnPT) in the development of antimicrobial textiles. ZnPT complex is used outside of textiles such as the treatment of dandruff, seborrheic dermatitis, and psoriasis in different areas. In recent years, different synthesis mechanisms of Ag-TiO_2 have been examined and it has been suggested that it can be used in different fields, especially due to its bactericidal activity [42]. There has been very little new study on the use of Ag-TiO_2 and ZnPT in the development of antimicrobial textile [19,43,44] and there are no detailed studies such as textile samples application, antimicrobial activity of the textile samples after application. In this context, in this study, fistly, it was determined that the two complexes have broad spectrum antimicrobial activity, and these complexes were applied to a textile sample containing 70% cotton and 30% polyester as a result of antimicrobial activity tests performed after the application; the most suitable temperature and time for the application of Ag-TiO_2 and ZnPT complexes was found as 50°C, and 60 min. The drying process was determined as 70°C for 60 min. It was observed that the antimicrobial activity of the ZnPT (≥0.005 g/l) complex and its durability (10 washes and 25 days of exposure in the open air) were much more active than the Ag-TiO_2 (≥0.5g/l) complex. Furthermore, textile samples applied with the two complexes were found to have high antimicrobial activity against all microorganisms including *P. vulgaris, E. cloacae, Y. pseudotuberculosis, L. monocytogenes, E. coli, S. typhimurium, S. epidermidis, E. faecalis, B. subtilis, K. pneumoniae, P. aeruginosa, S. aureus* and *C. albicans.* It can be said that the ZnPT complex, which gives the textile samples high antimicrobial activity in low concentration, is more effective.

The antimicrobial textile market size is estimated at $497.4 million in 2015 and is expected to reach $1.076.1 million, an increase of 7.4 percent from 2016 to 2026. This demand is expected to increase, especially for many products such as surgical materials, curtains, bedding, and upholstery. In this context, the use of the originally synthesized Ag-TiO_2 used in this study and ZnPT complex, which has the opportunity to be used in different sectors, in the development of antimicrobial textiles has been revealed. These products, which can meet the needs of such a developing sector, can be produced on a larger scale and brought to the industry and provide great export earnings. However, these processes on the fabric cause some changes in the fabric. In future studies, it is planned to apply these complexes to larger fabric surfaces and different fabric types, and then to reveal the changes in strength and fabric appearance. At the same time, it is planned to reveal the cost that may arise in the large-scale use of these complexes.

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