PREPARATION AND APPLICATION OF ORGANOPHOSPHORUS DIMERS AS ANTIMICROBIAL AGENTS FOR BAGASSE PACKAGING PAPER

MOHAMED EL-SAKHAWY, HASSAN M. AWAD,[*] HASSAN M.F. MADKOUR,[**] AHMED K. EL-ZIATY,[**] MONA ABDELKADER NASSAR[***] and SALAH A. A. MOHAMED[***]

Cellulose and Paper Department, National Research Centre, 33, El-Bohouth Str., Dokki, P.O. 12622, Giza, Egypt
[*]*Chemistry of Natural and Microbial Products Department, Pharmaceutical Industry Division, National Research Centre, 33, El-Bohouth Str., Dokki, P.O. 12622, Giza, Egypt*
[**]*Chemistry Department, Faculty of Science, Ain Shams University Abbassia, Cairo, Egypt*
[***]*Packing and Packaging Materials Department, National Research Centre, 33, El-Bohouth Str., Dokki, P.O. 12622, Giza, Egypt*
✉ *Corresponding author: M. El-Sakhawy, elsakhawy@yahoo.com*

Dedicated to the 70th anniversary of Acad. Bogdan C. Simionescu

Organophosphorus aniline and *p*-toluidine dimers were prepared and utilized as antimicrobial coating for bagasse paper sheets. Bagasse paper sheets coated with 1,3-diaryl-2,2,2,4,4,4-hexachlorocyclodiphosph(V)azane (where aryl is aniline or *p*-toluidine) demonstrated antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*), yeasts (*Candida albicans*, *Saccharomyces cerevisiae*) and fungi (*Aspergillus niger*). The antimicrobial activity attained for the bagasse paper sheets coated with the prepared organophosphorus dimers was attributed to the presence of chlorine and phosphorus atoms, in addition to the lone pair of electrons on nitrogen atoms, in the structure of the dimers. The optimum inhibitory activity of the bagasse paper sheets coated with organophosphorus dimers was obtained at 250 mg/mL, for the aniline dimer, and at 250-300 mg/mL, for the *p*-toluidine dimer, against most of the tested microorganisms. *p*-Toluidine was found to be superior to aniline in terms of their antimicrobial activity against the investigated microorganisms.

Keywords: antimicrobial activity, bagasse, paper sheets, coating, organophosphorus dimers

INTRODUCTION

Paper is the most commonly used packaging material worldwide. Paper and paperboards are commonly used in corrugated boxes, milk cartons, folding cartons, bags and sacks, as well as for wrapping.[1] Paper is made from natural and renewable resources and it has multiple advantages, such as low price, flexibility, printability, recyclability, good temperature resistance, excellent strength properties, good insulation properties, easiness in coating and lamination due to its compatibility with other packaging materials.[2] Depending upon the properties and basic weights, there are different types of paper and paperboards, which have gained wide application in packaging of numerous types of products. Food packaging can retard product deterioration, retain the beneficial effects of processing, extend shelf life, and maintain or increase the quality and safety of the packaged product.

The most frequent cause of food deterioration is microbial spoilage.[3] Recently, it has been suggested that using antimicrobial packaging materials is a potential way to ensure the protection of fresh foods and extend their shelf life. In antimicrobial packaging paper, antimicrobial substances are incorporated, or coated onto fibers to provide a barrier against microorganisms; in this manner, antimicrobial packaging prevents disease and spoilage.[4] A variety of barrier mechanisms have been suggested, including preventing access to the

product, preventing odor transmission and preserving the internal package atmosphere.[5]

Antimicrobial enhancement of paper sheets could be achieved by using silver-nanoparticles on acrylamide grafted bagasse paper.[6] Also, antimicrobial coating for packaging paper based on chitosan derivatives and quaternary ammonium salts immobilized on zinc oxide (ZnO) particles has been evaluated.[7] In addition, ZnO nanoparticles have been incorporated into polylactic acid to develop a nanocomposite coating layer for antimicrobial packaging application.[8] Natural essential oils[9] and nanocellulose/chitosan[10] were used as coatings to enhance the antimicrobial properties of packaging paper.

The majority of the antimicrobial polymeric materials investigated so far contain quaternary ammonium and/or phosphonium salts.[11] The negatively charged phospholipids in the cellular membranes of most bacteria are the target of cationic biocides, penetrating into the cell wall to destroy the cytoplasmic membrane, which causes the leakage of intracellular components and subsequent cell death.[11] The charged head groups of cationic biocidal polysaccharide ensure such penetration by interacting with the charges on the cell membranes. The neutralization of the surface charge of bacterial or fungal cell membranes disturbs the cell's selective permeability towards the external environment, which leads to the penetration of counter ions into the cellular cytoplasm, allowing their combination with the nucleic acids, which then leads to the microorganisms' death.[12]

Organophosphorus dimers have been found promising as eco-friendly flame retardants and plasticizers, and recently, their antifungal and antibacterial activities have been proven.[13] The aim of this work was to investigate the biological activity of bagasse paper sheets coated with newly prepared organophosphorous dimers. Then, the ability of the antimicrobial packaging paper to inhibit or reduce the growth of microbes, and thus enhance the safety and shelf life of packaged products, was assessed.

EXPERIMENTAL

Materials

Bagasse paper sheets without any surface treatment (from Edfu Co., Egypt) were used as paper substrates (blank). These papers were made from bleached bagasse kraft pulp, with a basic weight of 80 g/m^2 and a thickness of 0.1 mm. All the chemicals used were of analytical grade and used without further modification.

Synthesis of organophosphorus compounds

1,3-Diaryl-2,2,2,4,4,4-hexachlorocyclodiphosph(V) azane, where aryl is either aniline or *p*-toluidine, was prepared as described below.

Two different hexachlorocyclodiphosph(V)azanes were prepared essentially by the methods of Chapman,[14] in which phosphorus pentachloride in cold dry benzene reacted readily with aniline or p-toludene, to give 1,3-diaryl-2,2,2,4,4,4-hexachlorocyclo-diphosph(V)azane. A solution of aromatic amine (0.1 mole) in dry benzene (100 mL) was added dropwise to phosphorus pentachloride (20.9 g, 0.1 mole) in dry benzene (200 mL) at 15 °C for 1 hour. The reaction mixture was heated under reflux for three hours under anhydrous conditions. Then, the reaction mixture was cooled to room temperature and the formed solid was filtered and washed several times with dry benzene and then with dry diethyl ether. Hexachlorocyclo-diphosph(V)azane was obtained as solid crystals.

Coating experiments

Bagasse paper sheets were coated by immersing them into different concentrations of the dimers and then were left to air dry.

Antibacterial and antifungal activities

The antibacterial and antifungal activities of the bagasse paper sheets coated with aniline or *p*-toluidine dimer were investigated.

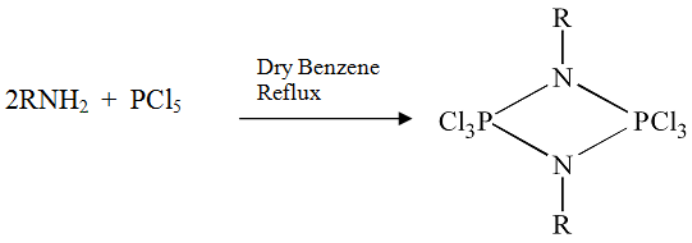


Figure 1: Preparation of 1,3-diaryl-2,2,2,4,4,4-hexachlorocyclodiphosph(V)azane

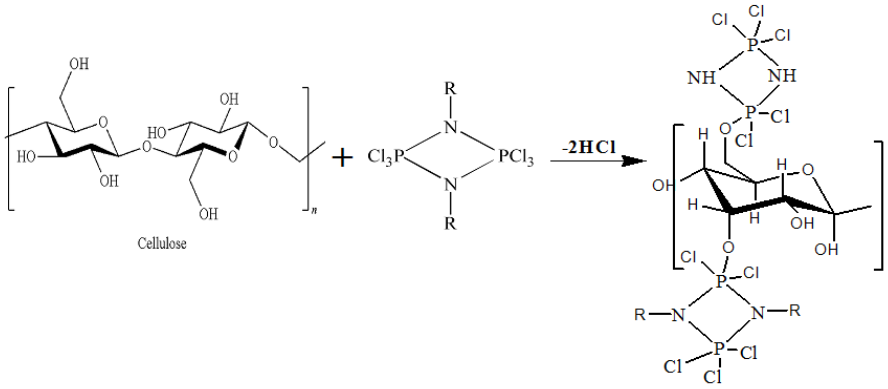


Figure 2: Reaction between cellulose and 1,3-diaryl-2,2,2,4,4,4-hexachlorocyclodiphosph(V)azane

Strains

The most frequently occurring pathogens and spoilage microorganisms in bakery products were selected. Antimicrobial activities were evaluated against Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*), yeasts (*Candida albicans*, *Saccharomyces cerevisiae*) and fungi (*Aspergillus niger*). The inhibition zones of microbial growth produced by different compounds of cyclodiphosph(V)azane derivatives, in different concentrations, were observed using the overlay method.[15]

Media used

The bacteria were slanted on nutrient agar (Merck, Darmstadt, Germany); the yeast was slanted and maintained on Sabaroud's agar medium (Lab M. Limited, Bury, Lancashire, UK); and the fungi were slanted and maintained on potato dextrose agar medium (Lab M. Limited).

Bioassays

The antimicrobial results were evaluated by the disk diffusion method.[16] The tested compounds were dissolved in dimethylformamide. The organisms were streaked in radial patterns on the plates. Then, the agar plates were incubated under aerobic conditions for 24 h and 48 h, at 37 °C and 28 °C for bacteria and fungi, respectively. All the samples were treated under the same conditions to obtain comparable results. Tests were carried out in three replicates. The plates were examined for evidence of antimicrobial activity, which was assessed by the inhibition zone of microbial growth around the paper disk.

RESULTS AND DISCUSSION

The equimolar ratios and the melting points of the prepared hexachlorocyclodiphosph(V)azane are presented in Table 1. Comparable values of the measured melting points of both aniline and *p*-toluidine dimers with those recorded in the literature verified the formation of the desired dimer with the empirical formula given in Table 1.[17] Different characterization techniques were used to elucidate the structure of the prepared dimers.[17]

Antimicrobial activity of prepared organophosphorus dimers

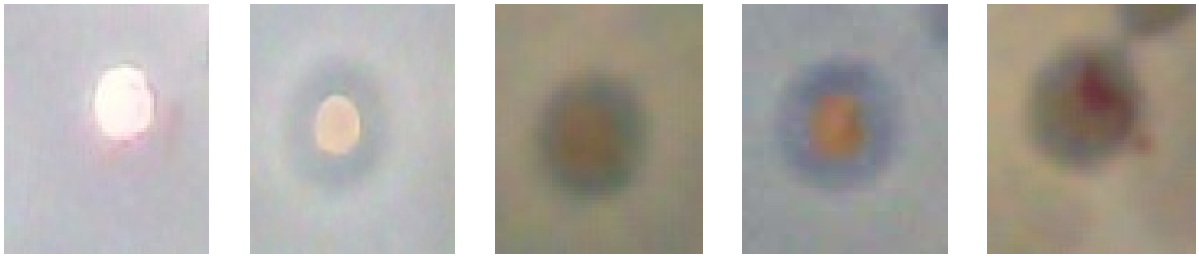
Organophosphorus compounds have been used as fire retardant, pesticides and nerve agents.[17] Recently, the biological activity of organophosphorus p-chloroaniline and p-anisidine dimers has been proven.[13]

Table 1
Equimolar ratio and melting points of hexachloro-cyclodiphosph(V)azane

Amino compound (g, mole)	Phosphorus pentachloride (g, mole)	Empirical formula	Melting point, °C	
			Measured	Literature
Aniline dimer (9.3; 0.1)	(20.9; 0.1)	$C_{12}H_{10}N_2P_2Cl_6$	179-182	180-182
p-toluidine dimer (10.7; 0.1)	(20.9; 0.1)	$C_{14}H_{14}N_2P_2Cl_6$	194-200	193-200

DMF as solvent | 200 mg against *S. aureus* | 200 mg against *E. coli* | 300 mg against *E. coli* | 300 mg against *P. vulgaris*

Figure 3: Antimicrobial activity of aniline coating



DMF as solvent | 150 mg against *S. aureus* | 300 mg against *P. aeruginosa* | 250 mg against *E. coli* | 250 mg against *C. albicans*

Figure 4: Antimicrobial activity of *p*-toluidine coating

In this study, the antimicrobial activity of the bagasse paper sheets coated with the prepared organophosphorus dimers can be attributed to the presence of Cl and P atoms, in addition to the lone pair of electrons on N atoms, in the dimers' structure.[13] Also, the phenolic fractions in the prepared aniline and *p*-toluidine dimers have the potential to inhibit the growth of Gram-negative bacteria by destroying their outer membrane, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to adenosine triphosphate.[18] The lone pair of electrons in the dimers' amino group has an essential role in their antimicrobial activity. It causes the leakage of intracellular components of the microorganisms by reacting with the negatively charged microbial cell membranes, in analogy to the action of chitosan.[19] Cyclodiphosphazanes, which show interesting properties, have been most intensively studied. Cyclodiphosph(V)azane, as a phosphorus–nitrogen compound, has been reported to inhibit the synthesis of proteins and thus to inhibit the growth of microorganisms.[20]

The antimicrobial activity of the prepared organophosphorus dimer coating on the surface of the paper sheets was expressed as the diameter (in millimeters) of clear inhibition zones of microbial growth.

Antibacterial and antifungal activity for 1,3-di-aniline 2,2,2,4,4,4-hexachlorocyclodiphosph(V) azane on bagasse paper

Antibacterial and antifungal activities of bagasse paper sheets coated with 1,3-di-aniline 2,2,2,4,4,4-hexachlorocyclodiphosph(V)azane, in different concentrations (from 100 to 300 mg solute/mL), were evaluated against Gram-positive bacteria and Gram-negative bacteria (Table 2), as well as against fungi (Table 3). The inhibition zones were classified as: weak (4.5-6 mm), moderate (6.1-7.9 mm) and significant (≥8 mm).

The results for the antibacterial and antifungal activities of bagasse paper sheets coated with 1,3-di-aniline 2,2,2,4,4,4-hexachlorocyclodi-phosph(V)azane are noted below. Ampicillin was used as a reference compound for evaluating the biocidal activity (Table 2).

The results obtained for Gram-positive bacteria are described below:

- *B. subtilis*: the concentrations of 200 and 250 mg/mL caused significant inhibition, compared to the reference compound, whereas the other studied concentrations showed moderate inhibition;
- *S. aureus*: all the concentrations investigated presented moderate inhibitory activity, compared to the reference compound, except the

concentration of 150 mg/mL, which showed weak inhibition.

As regards the activity of the developed coatings against Gram-negative bacteria, the results obtained in Table 2 signify the following:

- *P. aeruginosa*: all the concentrations showed weak inhibition, compared to the reference compound, except the concentration of 100 mg/mL, which did not show any inhibition at all;
- *E. coli*: the concentration of 300 mg/mL displayed significant inhibition and that of 250 mg/mL showed moderate inhibition, compared to the reference compound, whereas the concentrations of 200 and 150 mg/mL showed weak inhibition, and that of 100 mg/mL showed no inhibition at all, compared to the reference compound;
- *P. vulgaris*: the concentrations of 300 and 250 mg/mL presented moderate inhibitory activity, whereas the concentrations of 200 and 150 mg/mL showed weak inhibition. The blank sample and the concentration of 100 mg/mL caused no inhibition at all.

These results indicate that 250 mg solute/mL solvent was the optimum concentration for achieving significant inhibition results against Gram-positive bacteria and Gram-negative bacteria.

The synthesized compounds were also tested with regard to their antifungal activity and Mycostatin was used as a reference compound. The obtained results summarized in Table 3 reveal that:

- *C. albicans*: all the concentrations produced moderate inhibitory activity, except the concentration of 100 mg solute/mL, which did not show any inhibition at all;
- *S. cerevisiae*: the concentrations of 300 and 250 mg/mL led to significant inhibition compared to the reference compound, whereas other concentrations and the blank sample did not show any inhibition.

These results indicate that 250 mg solute/mL solvent was the optimum concentration for significant inhibition results against *C. albicans* and *S. cerevisiae*.

As regards the inhibition of *A. niger*, only the concentration of 200 mg solute/mL showed significant inhibition, whereas the other concentrations showed moderate antifungal activity compared to the reference compound. This makes the concentration of 200 mg solute/mL solvent the optimum one.

Table 2
Antibacterial activity of aniline dimer coating against Gram-positive and Gram-negative bacteria

Concentration, mg solute\mL solvent	Gram-positive bacteria		Gram-negative bacteria		
	B. subtitles	*S. aureus*	*P. aeruginosa*	*E. coli*	*P. vulgaris*
100	6.75	7.60	0.00	0.00	0.00
150	7.50	5.75	5.00	6.00	6.00
200	8.00	6.50	6.00	5.75	6.00
250	9.00	7.00	6.00	7.00	7.50
300	6.75	7.25	5.50	8.00	6.50
DMF	0.00	0.00	0.00	0.00	0.00

Table 3
Antifungal activity of aniline dimer coating

Concentration, mg solute\mL solvent	Fungi		
	Unicellular		Filamentous
	C. albicans	*S. cerevisiae*	*A. niger*
100	0.00	0.00	7.00
150	6.25	0.00	6.75
200	6.50	0.00	8.00
250	7.50	9.00	6.50
300	6.75	8.50	7.00
DMF	0.00	0.00	0.00

Table 4
Antibacterial activity of *p*-toluidine dimer coating against Gram-positive and Gram-negative bacteria

Concentration, mg solute\mL solvent	Gram-positive bacteria		Gram-negative bacteria		
	B. subtitles	*S. aureus*	*P. aeruginosa*	*E. coli*	*P. vulgaris*
100	7.00	8.50	5.00	5.50	0.00
150	0.00	14.50	5.00	5.25	5.50
200	7.00	7.75	7.25	7.00	9.50
250	9.00	8.75	7.00	10.00	8.50
300	8.00	20.00	9.00	10.50	8.00
DMF	0.00	0.00	0.00	0.00	0.00

Table 5
Antifungal activity of *p*-toluidine dimer coating

Concentration mg solute\mL solvent	Fungi		
	Unicellular		Filamentous
	C. albicans	*S. cerevisiae*	*A. niger*
100	5.00	0.00	12.00
150	6.00	0.00	8.00
200	7.75	9.00	8.00
250	9.50	10.50	8.00
300	9.50	10.50	8.00
DMF	0.00	0.00	0.00

Antibacterial and antifungal activities for 1,3-di-p-toluidine2,2,2,4,4,4-hexachlorocyclodiphosh(V)azane on bagasse paper

Antibacterial and antifungal activities of bagasse paper sheets coated with 1,3-di-*p*-toluidine 2,2,2,4,4,4-hexachlorocyclodiphosph(V)azane in different concentrations, from 100 to 300 mg solute/mL, were evaluated against Gram-positive and Gram-negative bacteria (Table 4), as well as against fungi (Table 5).

The results were assessed using ampicillin as reference antibiotic. The following findings have been revealed against Gram-positive bacteria (Table 4):

- *B. subtilis*: the concentrations of 300 and 250 mg/mL exhibited significant inhibition, compared to the reference compound, whereas the concentrations of 200 and 100 mg/mL showed moderate inhibition and that of 150 and the blank sample showed no inhibition at all. Thus, 250 mg/mL solvent is the most suitable concentration for achieving acceptable inhibitory results against *B. subtilis*.
- *S. aureus*: all the concentrations led to significant inhibition compared to the reference compound, except that of 200 mg solute/mL, which showed moderate inhibition, while the blank showed no inhibition at all. It is clear that 150 mg/mL solvent is the optimum concentration for a significant inhibition result against *S. aureus*, while the maximum inhibition result was attained at a concentration of 300 mg/mL solvent.

The results listed in Table 4 with regard to Gram-negative bacteria signify the following:

- *P. aeruginosa*: the concentration of 300 mg/mL caused significant inhibition, compared to the reference antibiotic, whereas the concentrations of 200 and 250 mg/mL showed moderate inhibition, those of 100 and 150 mg/mL presented weak inhibition, while the blank showed no inhibition at all;
- *E. coli*: the concentrations of 300 and 250 mg/mL showed significant inhibition compared to the reference antibiotic, that of 200 mg/mL showed moderate inhibition, and those of 100 and 150 mg/mL – weak inhibition, while the blank compound showed no inhibition. It is clear that the inhibition zones against *P. aeruginosa* and *E. coli* increased as the solute concentration was increased.
- *P. vulgaris*: the concentrations of 300, 250 and 200 mg/mL produced significant inhibition compared to the reference antibiotic, whereas the concentration of 150 mg/mL showed moderate inhibition and

that of 100 mg/mL and the blank compound showed no inhibition. This means that 200 mg/mL is the optimum concentration for significant inhibition results against *P. vulgaris*.

The synthesized compounds were tested with regard to their antifungal activity, using Mycostatin as a reference compound. The results obtained in Table 5 signify the following:

- *C. albicans*: the concentrations of 300 and 250 mg/mL showed significant inhibition compared to the reference antibiotic, that of 200 mg/mL showed moderate inhibition, and those of 150 and 100 mg solute/mL showed weak inhibition. The blank presented no inhibition at all. This means that 250 mg solute/mL solvent was the optimum concentration.
- *S. cerevisiae*: the concentrations of 300, 250 and 200 mg/mL showed significant inhibition, compared to the reference antibiotic, whereas those of 150, 100 mg/mL and the blank showed no inhibition. An acceptable inhibition result was attained at 200 mg/mL solvent (9.0 mm), while the maximum inhibition was attained at 250 mg/mL solvent (10.5 mm).
- *A. niger*: all the concentration levels showed significant antifungal activity, compared to the reference compound, while the blank showed no inhibition, as expected. For economical considerations, the optimum concentration can be regarded that of 100 mg solute/mL solvent.

Table 6 tabulates the concentrations of the organophosphorus aniline or *p*-toluidine dimers used to coat bagasse paper sheets that gave the highest inhibition zones. Table 6 and Figure 5 reveal that the highest inhibition zone was obtained at 250 mg/mL of aniline dimer against most microorganisms, except *S. aureus*, *P. aeruginosa*, *E. coli* and *A. niger*, for which high inhibition zones have been obtained at 100, 200, 300 and 200 mg/mL, respectively.

Table 6
Optimum inhibition results for bagasse paper sheets treated with dimers

Microorganism	Aniline		*p*-Toluidine	
	High inhibition zone (mm)	At mg/ml conc.	High inhibition zone (mm)	At mg/ml conc.
B. subtitles	9.00	250	9.00	250
S. aureus	7.60	100	20.00	300
P. aeruginosa	6.00	200	9.00	300
E. coli	8.00	300	10.50	300
P. vulgaris	7.50	250	9.50	200
C. albicans	7.50	250	9.50	250
S. cerevisiae	9.00	250	10.50	250
A. niger	8.00	200	12.00	100

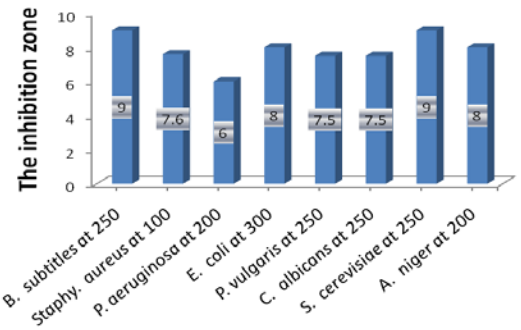


Figure 5: Optimum results for paper sheets treated with aniline dimer

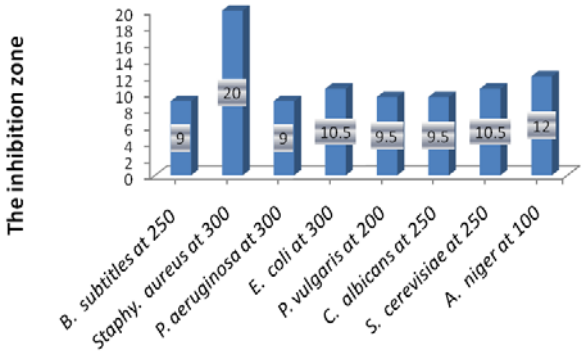


Figure 6: Optimum results for paper sheets treated with *p*-toluidine dimer

Figures 5 and 6 show the highest inhibition zone obtained for bagasse paper sheets treated with aniline and *p*-toluidine, respectively, at optimum concentrations against most microorganisms.

The highest inhibition zones produced by the coating containing the *p*-toluidine dimer (Fig. 6) were noted for the concentration of 300 mg/mL against *S. aureus*, *P. aeruginosa* and *E. coli*, and the concentration of 250 mg/mL against *B. subtitles*, *C. albicans* and *S. cerevisiae*. Lower concentrations were sufficient for biocidal activity against *P. vulgaris* (200 mg/mL) and *A. niger* (100 mg/mL).

By comparing the results obtained in this study with regard to the biocidal action of organophosphorus aniline and *p*-toluidine dimers with previously reported ones concerning the effects of p-chloroaniline and p-anisidine dimers,[13] it could be concluded that the investigated dimers exert antimicrobial activity in the following order: *p*-toluidine > p-chloroaniline > p-anisidine > aniline.

CONCLUSION

The present study investigated the biological activity of bagasse paper sheets coated with newly prepared organophosphorous dimers: aniline and *p*-toluidine. It was found that the best inhibitory activity of the coated bagasse paper sheets can be obtained at a concentration of 250 mg/mL of aniline against most microorganisms, except *S. aureus*, *P. aeruginosa*, *E. coli* and *A. niger*, for which the highest inhibition zones were achieved with concentrations of 100, 200, 300 and 200 mg/mL, respectively. Meanwhile, the *p*-toluidine coating presented the strongest inhibitory activity at 250-300 mg/mL concentration against most of the tested microorganisms, except *P. vulgaris* and *A. niger*, for which lower concentrations, of 200 mg/mL and 100 mg/mL, respectively, were sufficient. Also, it can be concluded that the biocidal action of the *p*-toluidine coating is much superior to that of the aniline one against the investigated microorganisms.

ACKNOWLEDGEMENT: The authors express their deep gratitude to the National Research Center for the financial support for this work.

REFERENCES

[1] D. Raheem, *Emir. J. Food Agric*., **25**, 177 (2012).
[2] H. M. El-Sherif, A. M. Nasser, A. I. Hussin, H. Abd El-Wahab, M. B. M. Ghazy *et al*., *Egypt. J. Chem*., **59**, 935 (2016).
[3] L. C. Cabral, V. F. Pinto and A. Patriarca, *Int. J. Food Microbiol*., **166**, 1 (2013).
[4] L. Vikele, M. Laka, I. Sable, L. Rozenberga, U. Grinfelds *et al*., *Cellulose Chem. Technol*., **51**, 67 (2017).
[5] K. Marsh and B. Bugusu, *J. Food Sci*., **72**, R39 (2007).
[6] S. Kamel, *Carbohyd. Polym*., **90**, 1538 (2012).
[7] P. Nechita, E. Bobu, G. Parfene, R. M. Dinică and T. Bălan, *Cellulose Chem. Technol*., **49**, 625 (2015).
[8] H. Zhang, M. Hortal, M. Jordá-Beneyto, E. Rosa, M. Lara-Lledo *et al*., *LWT - Food Sci. Technol*., **78**, 250 (2017).
[9] A. Rodríguez, R. Batlle and C. Nerín, *Prog. Org. Coat*., **60**, 33 (2007).
[10] M. A. El-Samahy, S. A. A. Mohamed, M. H. Abdel Rehim and M. E. Mohram, *Carbohyd. Polym*., **168**, 212 (2017).
[11] Y. Xue, H. Xiao and Y. Zhang, *Int. J. Mol. Sci*., **16**, 3626 (2015).
[12] M. F. Zaky, A. M. Badawi, I. A. Sabbah, R. A. Abd Elghani, E. A. Ismaeil *et al*., *Egypt. J. Chem*., **55**, 259 (2012).
[13] M. El-Sakhawy, H. M. Awad, H. M. F. Madkour, A. K. El-ziaty, M. A. Nassar *et al*., *Int. J. Technol*., **6**, 932 (2016).
[14] A. C. Chapman, W. S. Holmes, N. L. Paddock and H. T. Searle, *J. Chem. Soc*., **Resumed**, 1825 (1961).
[15] M. Hayakawa, K. Ishiwwa and H. Nonomurha, *J. Ferm. Technol*., **66**, 367 (2004).
[16] S. Ganapathy and S. Karpagam, *J. Pharmacogn. Phytochem*., **5**, 253 (2016).
[17] S. A. Mohamed, M.Sc. Thesis, Faculty of Science, Al-Azhar University, Cairo, 2006, p. 212.
[18] E. Kusrini, A. Usman, C. D. Wisakanti, R. Arbianti and D. A. Nasution, *Makara J. Technol*., **19**, 148 (2015).
[19] L. A. M. van den Broek, R. J. I. Knoop, F. H. J. Kappen and C. G. Boeriu, *Carbohyd. Polym*., **116**, 237 (2015).
[20] A. N. M. A. Alaghaz, *J. Mol. Struct*., **1068**, 27 (2014).