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Antimicrobial and biological effects of N-diphenylphosphoryl-P-triphenylmonophosphazene-II and di(*o*-tolyl)-phosphoryl-P-tri(*o*-tolyl)monophosphazene-III on bacterial and yeast cells

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Abstract

The purpose of the study was to synthesize and evaluate the antimicrobial effects of two monophosphazenes, N-diphenylphosphoryl-P-triphenylmonophosphazene-II and N-di(*o*-tolyl)phosphoryl-P-tri(*o*-tolyl)monophosphazene-III on bacterial and yeast strains. The biological effects of these molecules were compared with a potential antioxidant vitamin E. According to results, the triphenyl monophosphazene-II has antimicrobial effect on all the bacterial and yeast cells, but tri(*o*-tolyl)monophosphazene-III has only antimicrobial effect on some bacterial cells. When the concentration of triphenyl monophosphazene-II was raised, it was observed that inhibition zone increased on the bacterial growth media. The biological effects of these molecules were compared to vitamin E in the *Saccharomyces cerevisiae* culture media. In 200 μg administered culture media, the cell density decreased in vitamin E, triphenyl monophosphazene-II and tri(*o*-tolyl)monophosphazene-III groups at the end of 24 and 48 h incubation times ($p < 0.001$, $p < 0.05$). While the cell densities in vitamin E

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and tri(*o*-tolyl)monophosphazene-II groups decreased partly at the end of 72 h incubation time ($p < 0.05$), its level in triphenyl monophosphazene-II group increased ($p < 0.01$) at the same incubation time. In 1000 μg administered culture media, cell density was not found to differ between vitamin E and control groups at the end of 24 h incubation time, but it was found that the cell densities in triphenyl monophosphazene and tri(*o*-tolyl)monophosphazene-III groups decreased at the same incubation time ($p < 0.001$). The cell densities in tri(*o*-tolyl)monophosphazene-III group and triphenyl monophosphazene-II decreased at the end of 48 h incubation time (respectively, $p < 0.05$, $p < 0.001$). In 200 μg administered cell pellets, while the lipid level was not found to differ between control and vitamin E, the lipid level decreased in triphenyl monophosphazene-II and tri(*o*-tolyl)monophospazene-III groups (respectively, $p < 0.001$, $p < 0.01$). In 1000 μg administered cell pellets, it was found that the lipid level decreased in vitamin E, triphenyl monophosphazene-II and tri(*o*-tolyl)monophosphazene-III groups ($p < 0.001$, $p < 0.01$).

Keywords: Triphenyl monophosphazene-II; Tri(*o*-tolyl)monophosphazene-III; Antimicrobial and biological effects; Vitamin E; Bacterial and yeast cells

1. Introduction

Phosphazenes are compounds which contain alternating phosphorus and nitrogen atoms in their skeleton. These compounds fall into three categories: the cyclo, the monophosphazenes, and polyphosphazenes. The cyclo and polyphosphazenes are probably the best known and most intensively studied phosphorus–nitrogen derivatives. Monophosphazenes have the structure $RN{=}PR_3$. They show many fundamental similarities to the cyclic and polymeric phosphazenes. They are particularly interesting from the view point of their bonding structure and much of the physical–inorganic work carried out on these compounds has an important bearing on the skeletal bonding found in cyclic and polymeric phosphazenes [1,2].

Phosphazenes possess a number of characteristics such as use as drug components for chemotherapeutic applications, and antibacterial activity. These compound are used in the structure of medical implants and drug delivery systems. In such applications where the polymer serves a temporary function, the ultimate degradability of the material to harmless fragments is a prerequisite for successful medical use. Phosphazene compounds and derivatives are widely used against bacterial agents in medicine on the surface cover in cardiovascular devices, knee and hip joints, and in ocular lens. These compounds covalently bind to the surface or with their side groups bind strongly to surface through ionic and hydrogen binding or van der Waals forces. Maximum interactions with microorganisms would be expected for polymer chain sites of a soluble macromolecule that is anchored to a surface [2–5].

Phosphazene polymers with an amino acid ester as cosubstituents are potentially biodegradable in vivo. Polyphosphazenes with amino acid groups are flexible,

generally biodegradable, and erode hydrolytically to the amino acid, phosphate, and ammonia. Also, their derivatives have been stated to be biodegradable and biocompatible molecules. Consequently these compounds are of potential interest as biomaterials [2–4].

Vitamin E is the major lipid-soluble antioxidant present in blood and cell membranes of higher organisms. It acts synergistically with other antioxidants in cells, to protect them from damage and lyses induced by oxidative stress [6]. Tocopherols can undergo oxidation–reduction reactions on the aromatic ring [7,8]. The vitamin activity of tocopherols likely results from their ability to prevent oxidative damage to the lipids of cellular membranes [9–12].

Veromese et al. [13] have stated that antibacterial or anti-inflamatory drugs are used in periodontal tissue regeneration and released both in vitro and in vivo at a rate that ensured therapeutic concentration in surrounding tissue. Öztürk et al. [14] found that trichlorophosphazene, 2-isopropiloxyetoxy monophosphazene, and allyloxy monophosphazene have antimicrobial effect in microbial growth media. But, they have stated that the cell density of yeast cell in vitro media have been increased by these compounds. In addition, benzyloxy monophosphazene and phurphuryloxy monophosphazenes have antimicrobial effect in microbial growth media [15]. But, these compounds increased the cell densities of yeast cell in vitro media. It has been stated that the phurphuryloxy monophosphazene and vitamin E have decreased lipid level in cell pellet of *Saccharomyces cerevisiae* [15]. In addition, the lipid level of *S. cerevisiae* was decreased by isopropiloxyetoxy monophosphazene and allyloxy monophosphazene compounds.

In this study, it was proposed to synthesize and evaluate the antimicrobial effects of two monophosphazenes, N-diphenylphosphory-P-triphenylmonophosphazene-II and N-di(*o*-tolyl)phosphoryl-P-tri(*o*-tolyl)monophosphazene-III on the bacterial and yeast stains. In addition, the biological effects of these molecules on the lipid level of *S. cerevisae* were compared with a potential antioxidant vitamin E.

2. Materials and methods

2.1. Materials

Organic solvents used in experimental work were dried by conventional methods. Using Kieselgel 60F254 (silica gel) precoated TLC plates, all reactions were monitored and the separating conditions were determined. Separation of products was carried out by flash column chromatography using Kieselgel 60.

The purity of each monophosphazene compound was established by column chromatography, NMR, mass spectroscopy; and elemental analysis. IR spectra were recorded with a MIDIAC 1700 M FTIR spectrometer. ^{1}H and ^{13}C-NMR spectra were recorded using a GEMINI VARIAN 200 spectrometer. ^{31}P-NMR spectra were recorded. FISONS EA 1108 and LECO 932 CHNS-O elemental analysis apparatus was used for microanalysis.

2.2. Synthesis of monophosphazenes

To synthesize N-diphenylphosphoryl-P-triphenylmonophosphazene, 150 ml $NP_2O(Cl_5)$ (0.005 mol, 1.46) was slightly dropped to 13.50 ml C_6H_6MgCl (dissolved in 2 M tetrahydrofuran) in room temperature [16]. Then, the mixture was refluxed under back cooling for 24 h. At the end of reaction, salt product was separated by filtration and the solvent mixture was rotary evaporated. Then the product of reaction was dissolved in chloroform:acetone (2:1, v/v) and purified in silica gel column. The Rf value of product was determined as 0.51 and mp 150 °C (Figs. 1 and 2, Table 1).

To synthesize N-di(*o*-tolyl)phosphoryl-P-tri(*o*-tolyl)monophosphazene, 150 ml $NP_2O(Cl_5)$ was slightly dropped to 22 ml *o*-$CH_3C_6H_4$ MgCl (dissolved in 1 M tetrahydrofuran) in room temperature. Then, the mixture was refluxed under back cooling for 24 h. At the end of reaction, salt product was separated by filtration and the solvent mixture was rotary evaporated. Then, the product of reaction was dissolved in chloroform:acetone (4:1, v/v) and purified in silica gel column. The Rf value of product was determined as 0.63 and m.p. 145 °C.

2.3. Bacterial and yeast strains

Bacterial cells: *Listeria monocytogenes SCOTT A*; *Bacillus megaterum DSM 32*; *Escherichia coli ATCC 25922*; *Enterobacter aeroginase CCM 2531*; *Bacillus brevis*

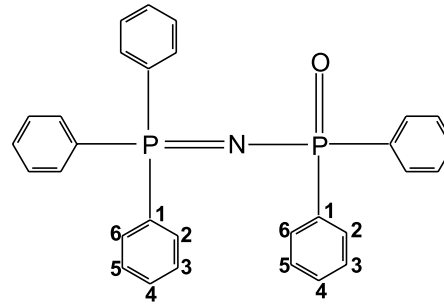

Fig. 1. N-diphenylphosphoryl-P-triphenylmonophosphazene-(II).

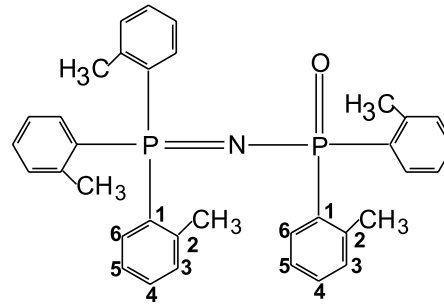

Fig. 2. N-di(o-tolyl)phosphoryl-P-tri(o-tolyl)monophosphazene-(III).

Table 1
The characterization of N-diphenylphosphoryl-P-triphenylmonophosphazene-(II)

δ (ppm)	J (Hz)	Proton type
^{1}H NMR		
7.13–7.20	1.9	*J*PCCCCH P=N, P=O (**4**, **4**)
7.28–7.33	4.7	*J*PCCCH P=N (**3**, **5**)
7.39–7.43	6.0	*J*PCCCH P=O (**3**, **5**)
7.61–7.64	7.20	*J*PCCH P=N (**2**, **6**)
7.69–7.72	6.60	*J*PCCH P=O (**2**, **6**)
^{13}C NMR		
128.0	12.6	*J*PCC P=O (**2**, **6**)
128.9	12.8	*J*PCC P=N (**2**, **6**)
130.2	2.4	*J*PCCCC P=O (**4**)
130.0 and 130.5	51.2	*J*PC P=O (**1**)
131.6	10	*J*PCCC P=O (**3**, **5**)
132.3	2.8	*J*PCCCC P=N (**4**)
133.0	11.1	*J*PCCC P=N (**3**, **5**)
138.0 and 140.0	173.2	*J*PC P=N (**1**)
^{31}P NMR		
15.45	–	P=N
14.38	–	P=O

FMC 3; *Micrococcus luteus*, *Pseudomonos vulgaris*, and *Staphylococcus aureus COVAN 1*; *Yeast cells*: *S. cerevisiae FMC 16*; *Candida albicans FMC 17*.

2.4. Preparation of microbial culture media

Bacterial strains were inoculated to Nutrient Buyyon culture (Difco) and incubated at 30 ± 0.1 °C for 24 h. Yeast cells were inoculated into Malt Extract Buyyon (Difco) and incubated at 30 °C for 48 h. To test the antimicrobial effects, triphenyl monophospahazene, and tri(*o*-tolyl)monophospahazene the 15 ml Hilton agar was placed in petri dishes and the strains of bacterial and yeast were inoculated 100 μl into culture media from cell culture. After agar hardened, disks were placed on the agar of 6-mm diameter and to which monophosphazene compounds were absorbed. Then, petri dishes were left at the 4 °C for 2 h and these cultures were incubated at 35 ± 0.1 °C for 18–24 h and yeast cultures were incubated at 30 ± 0.1 °C for 72 h. At the end of incubation time, the inhibition zones on the bacterial and yeast nutrient media were measured in millimeters [14,15].

2.5. Preparation of disks

To prepare the disks, the triphenyl monophospahazene and tri(*o*-tolyl)monophospahazene compounds were dissolved in toluene and absorbed into disks which are made from filter paper, by using micro injector. In addition, purified toluene was absorbed onto control disks.

2.6. Preparation of yeast strain growth media

The strains of *S. cerevisiae* were grown in YDP medium. One percent (w/v) yeast extract (Merck), 2% (w/v) bactopeptone (Acumedia), 2% glucose (w/v) (Sigma, St. Louis, MO). After the growth media had been prepared, their turbidites were measured 550 nm by using an Ultra spectrophotometer. Then, cultures were divided into groups as control, triphenyl monophosphazene-II, tri(*o*-tolyl)monophosphazene-III, and vitamin E. Then, each group was administered 200 and 1000 μg monophosphaze compound and vitamin E and yeast cells were inoculated 100 μl into culture media from the previous yeast cultures. After the inoculation, cells densities in the growth media of *S. cerevisiae* were measured at 550 nm for 24, 48, and 72 h. At the end of incubation time, cells were collected by centrifugation at 4500 rpm for 10 min and pellets were washed three times with 0.05 M potassium phosphate buffer (pH = 6.8). Cell pellets were maintained at −22 °C until the lipid analysis.

2.7. Lipid extraction

The total lipids were extracted with chloroform–methanol (2:1, v/v) by the method of Folch et al. [17] as previously described [18]. The cell pellets of *S. cerevisiae* were taken and homogenized with the mixture of chloroform–methanol (2:1, v/v) in a test tube with Teflon flask. Non-lipid contaminants were removed by washing with 0.88% KCl solution. The solvent of extracts was evaporated in a rotary evaporator at 45 °C by using vacuum and lipid residue was dissolved in purified hexane and stored at −22 °C.

2.8. Determination of total lipids

The total lipids were determined by the method Fring et al. [19] as previously described [14,15,20]. Two hundred microliters of each solution from extracted lipids was treated with 500 μl of concentrated sulphuric acid and left in boiling water for 10 min. Then, 5 ml of phosphovanilin reagent was added and the mixture was incubated at 25 °C for 20 min. After incubation the absorbency of samples was read at 540 nm against a blank. To calculate the level of lipids of cell pellets, standard curve was prepared in the same conditions from USA grade of olive oil (Sigma, St. Louis, MO).

2.9. Statistical analysis

Values were reported as means ± SD. Statistical analysis was performed using SPSS Software. The variance analysis (ANOVA) and LSD test were used for comparison between the control group and administered groups.

3. Results

3.1. The antimicrobial and biological effects of monophosphazenes

While the triphenyl monophosphazene-II has antimicrobial effect on the all bacterial and yeast cells in the study, tri(*o*-tolyl)monophosphazene-III has only

antimicrobial effect on the some bacterial cells (Tables 2 and 3). When the concentration of triphenyl monophosphazene-II was raised, it was observed that inhibition zone increased on the bacterial growth media. But, tri(*o*-tolyl)monophosphazene compound has no such effect on the bacterial cells. In addition, when the tri(*o*-tolyl) monophosphazene-III was used in 250 μg dose, it was observed that it has no antimicrobial effect on the bacterial and yeast cells (Table 3).

The biological effects of these compounds were compared to vitamin E on the *S. cerevisiae* culture media. In 200 μg administered culture media, the cell density decreased in vitamin E, triphenyl monophosphazene-II, and tri(*o*-tolyl)monophosphazene-III groups, compared with control group at the end of 24 h incubation time ($p < 0.001$). While the cell density decreased significantly in tri(*o*-tolyl)monophosphazene-III group at the end of 48 h incubation time ($p < 0.001$), the cell density decreased partly in triphenyl monophosphazene-II group ($p < 0.05$). Also, it was observed that the cell density in vitamin E group decreased in the same incubation

Table 2
The characterization of N-di(*o*-tolyl)phosphoryl-P-tri(*o*-tolyl) monophosphazene-(III)

δ (ppm)	ΔJ (Hz)	Proton type
^{1}H NMR		
2.04	–	–CH_3 P=N
2.13	–	–CH_3 P=O
7.33	4.5	*J*PCCCCH P=O **(4)**
6.87–6.89	6.1	*J*PCCCH P=O, P=O **(3)**
6.98–6.99	7.3	*J*PCCCCH P=N **(5)**
7.06–7.09	7.4	*J*PCCCCH P=N **(4)**
7.11–7.15	7.6	*J*PCCCH P=N **(5)**
7.63–7.69	7.67	*J*PCCH P=N **(6)**
7.69–7.74	7.57	*J*PCCH P=O **(6)**
^{13}C NMR		
21.8	3.89	–CH_3 P=O
22.9	4.36	–CH_3 P=N
124.9	12.2	*J*PCC P=O **(6)**
126.2	13.3	*J*PCC P=N **(6)**
128.5 and 129.5	101.2	*J*PC P=O **(1)**
130.2	1.8	*J*PCCC P=O **(4)**
131.2	11.8	*J*PCCC P=O **(3)**
132.3	2.6	*J*PCCC P=N **(4)**
132.5	8.8	*J*PCCC P=N **(3)**
133.2	9.6	*J*PCCCC P=O **(5)**
134.9	130.5	*J*PCCCC P=O **(5)**
138.1 and 136.5		*J*PC P=N **(1)**
141.5	11	*J*PCC P=O **(2)**
142.9	9.2	*J*PCC P=N **(2)**
^{31}P NMR		
14.63	–	P=O
14.84	–	P=N

Table 3
The antimicrobial effects of N-diphenylphosphoryl-P-triphenylmonophosphazene-II and N-di(*o*-tolyl)phosphoryl-P-tri(*o*-tolyl)monophosphazene-III on the bacterial and yeast cells (inhibition zone: mm)

	250 II*	500 II*	1000 II*	250 III*	500 III*	1000 III*
Bacterial strains						
Listeria monocytogenes	11	11	11	–	–	–
Bacillus megaterum	11	11	11	–	–	–
Escherichia coli	11	12	12	–	8	8
Enterobacter aeroginase	10	10	11	–	–	–
Bacillus brevis	10	10	13	–	–	–
Micrococcus luteus	12	12	13	–	8	8
Pseudomonos vulgaris	12	12	12	–	–	–
Staphylococcus aureus	10	10	12	–	–	–
Yeast strains						
Saccharomyces cerevisiae	9	10	11	–	8	8
Candida albicans	18	18	18	–	–	9

*II, N-diphenylphosforyl-P-triphenylmonophosphazene-II; **III, N-di(*o*-tolyl)phosphoryl-P-tri(*o*-tolyl)monophosphazen-III.

time. The cell densities in vitamin E and tri(*o*-tolyl)monophosphazene-II groups decreased partly at the end of 72 h incubation time ($p < 0.05$), but the cell density in triphenyl monophosphazene-II group increased in comparison to control ($p < 0.01$) at the end of the same time (Table 4).

In 1000 μg administered culture media, the cell density was not found to differ between control and vitamin E groups at the end of 24 h incubation time, but it was determined that the cell densities in triphenyl monophosphazene and tri-(*o*-tolyl)monophosphazene-III groups decreased at the end of the same time ($p < 0.001$). The cell densities in tri(*o*-tolyl)monophosphazene-III and triphenyl

Table 4
The variation of cell densities in 200 μg growth media of *S. cerevisiae* on during the incubation time

Groups	Culture media	After 24 h	After 48 h	After 72 h
Control	0.174 ± 0.03	1.435 ± 0.10	2.080 ± 0.07	2.320 ± 0.04
Vitamin E	0.183 ± 0.03	1.231 ± 0.05^{a}	1.880 ± 0.08^{b}	2.232 ± 0.07^{c}
II*	0.185 ± 0.05	1.147 ± 0.01^{a}	2.025 ± 0.05^{c}	2.405 ± 0.05^{b}
III**	0.165 ± 0.03	1.058 ± 0.07^{a}	1.596 ± 0.09^{a}	2.175 ± 0.05^{b}

*II, N-diphenylphosforyl-P-triphenylmonophosphazene-II; **III, N-di(*o*-tolyl)phosphoryl-P-tri(*o*-tolyl)monophosphazen-III.
[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$.

monophosphazene-II groups decreased at the end of 48 h incubation time (respectively, $p < 0.05$, $p < 0.001$). No differences were found among the control, vitamin E, and monophosphazene groups at the end of 72 h incubation time (Table 5).

At the end of incubation time, it was evaluated that the lipid level in cell pellets of *S. cerevisiae*. In 200 μg administered cell pellets, no differences were found between control and vitamin E, but it was observed that the lipid level decreased in triphenyl monophosphazene-II and tri(*o*-tolyl)monophospazene-III groups (respectively, $p < 0.001$, $p < 0.01$) (Table 6). In 1000 μg administered cell pellets, it was found that the lipid level decreased in vitamin E, triphenyl monophosphazene-II, and tri(*o*-tolyl) monophosphazene-III groups ($p < 0.001$, $p < 0.01$) (Table 6).

4. Discussion

The triphenyl monophosphazene-II compound showed antimicrobial activity on the bacterial and yeast cells in 250, 500, and 1000 μg concentrations. When the

Table 5
The variation of cell densities in 1000 μg growth media of *S. cerevisiae* on during the incubation time

Groups	Culture media	After 24 h	After 48 h	After 72 h
Control	0.154 ± 0.05	1.435 ± 0.10^{a}	2.080 ± 0.07^{a}	2.320 ± 0.04^{a}
Vitamin E	0.174 ± 0.05	1.486 ± 0.02^{a}	2.104 ± 0.02^{a}	2.250 ± 0.02^{a}
II*	0.166 ± 0.04	1.220 ± 0.02^{b}	1.757 ± 0.12^{c}	2.318 ± 0.03^{a}
III**	0.164 ± 0.03	1.201 ± 0.03^{b}	1.982 ± 0.09^{d}	2.295 ± 0.07^{a}

*II, N-diphenylphosforyl-P-triphenylmonophosphazene-II; **III, N-di(*o*-tolyl)phosphoryl-P-tri(*o*-tolyl)monophosphazen-III.
[a] $p > 0.05$.
[b] $p < 0.001$.
[c] $p < 0.01$.
[d] $p < 0.05$.

Table 6
The effects of N-diphenylphosphoryl-P-triphenylmonophosphazene-II vs N-di(*o*-tolyl)phosphoryl-P-tri(*o*-tolyl)monophosphazene-III on the lipid level of *S. cerevisiae* (mg/g wet cell pellet)

Groups	200 μg	1000 μg
Control	7.34 ± 0.23^{a}	7.84 ± 0.44
Vitamin E	7.45 ± 0.13^{a}	3.55 ± 0.23^{b}
II*	4.60 ± 0.05^{b}	5.11 ± 0.09^{c}
III**	5.18 ± 0.14^{c}	3.55 ± 0.23^{b}

*II, N-diphenylphosforyl-P-triphenylmonophosphazene-II; **III, N-di(*o*-tolyl)phosphoryl-P-tri(*o*-tolyl)monophosphazene-III.
[a] $p > 0.05$.
[b] $p < 0.001$.
[c] $p < 0.01$.

concentration of this compound in disk was increased, its antimicrobial effect increased partly on some bacterial and *S. cerevisiae* (Table 1). The antimicrobial effect of this compound may be due to the phenyl ring in its structure skeleton because phenol ring has an antibacterial effect on the microbial growth media [2]. In addition, tri(*o*-tolyl)monophosphazene-III has partly an antimicrobial activity on some bacterial and yeast cells in 500 and 1000 μg concentrations (Table 3). The antimicrobial activity of this compound may due to toluene ring in its molecular skeleton (Fig. 1).

Allcock et al. [2] had examined the antimicrobial effects of water-soluble phosphazene high polymer on the bacterial strains. They found that some compounds have antibacterial effect on some bacterial strains at 50,000 μg/ml. As the concentration of compounds was decreased, it was observed to decrease activity on the bacterial strains. Öztürk et al. [14] found that allyloxy monophophazene compound has antimicrobial effect on the bacterial cells at least at the 600 μg concentration, but isopropyloxy monophosphazene has antimicrobial effect in the 1000 μg concentration. Konar et al. [15] found that benzyloxy monophosphazene has antimicrobial effect on the bacterial and yeast strains among the 100–1500 μg concentrations, but phurpuryloxy monophosphazene has only on some bacterial and yeast cells in 1500 μg. These results indicate that the monophosphazene molecules have an antimicrobial effect on bacterial and yeast cells. Allcock et al. [2] observed that water-soluble phophazene compounds in dilution have no activity only on the bacterial cells, but dilute ampilicin and phenol solutions still possessed significant activity. These authors suggested that the use of these phosphazene molecules may be an advantage despite their lower activity. Our results showed that while the triphenyl monophosphazene-II has antimicrobial effect on the bacterial and yeast cells, tri(*o*-tolyl)monophospahezene-III has lower antimicrobial effects on the same microorganism. However, the tri(*o*-tolyl)monophospahezene-III may act against the bacterial agents despite their lower effect. Also, these monophosphazenes may be used in the structure of medical implants such as cardiovascular devices, knee and hip joints, and intraocular lens as covering material.

In in vitro experiments, the biological effects of these monophosphazene compounds were compared with the powerful antioxidant vitamin E. In 200 μg administered culture media, the cell density in triphenyl monophosphazene-II group only increased compared with control at the end of 72 h incubation time (Table 4). But, vitamin E decreased the cell density in growth media at the end of 24, 48, and 72 h. Öztürk et al. [14] and Konar et al. [15] found that some monophosphazene compounds increased the cell densities in growth media of yeast cells. In contrast, these authors have stated that these monophosphazene compounds have an antimicrobial effect on the microbial growth media.

In 1000 μg administered culture media, it was determined that the cell densities in triphenyl monophosphazene and tri(*o*-tolyl)monophosphazene-III groups decreased at the end of the 24 and 48 h. The decreasing of the cell density in triphenyl monophospazene-II and tri(*o*-tolyl)monophospazene-III groups can be due to their powerful molecular skeleton. But this molecular skeleton can be degradable by cells in time.

Triphenyl monophosphazene-II increased cell density in *S. cerevisiae* growth media compared with the control. The increase of the cell densities in this group

can be either due to use as a carbon source or from their biological effects. Although it has been reported that the most important carbon sources for fungal growth are glucose and maltose, alkanes can be carbon sources for fungal cells. Also, the fungal growth requires some compounds containing phosphorus and nitrogen atoms [21]. The monophosphazene molecules contain phosphorus and nitrogen atoms and therefore they have a positive effect in the growth media of yeast cells.

Ibim et al. [21] and Laurencin et al. [22] indicated that polyphosphazenes have positive effects on cells and tissue cultures and are required for cell growth and tissue regeneration. Also, Veromese et al. [13] found that antibacterial drugs, useful in periodontal tissue regeneration, could be entrapped in the phosphazene membranes and released both in vitro and in vivo at a rate that ensured therapeutic concentrations in the surrounding tissues. In our study, we obtained similar results with triphenyl monophosphazene in culture of *S. cerevisiae*. Triphenyl monophosphazene has an antimicrobial effect on the growth media of bacterial and yeast cells, but it has increased the cell densities in in vitro media at the end of 72 h incubation time. The increasing of the cell densities in these groups can be due to biodegradable compounds. Allkock et al., Crommen et al., Ruiz et al. [2–5] reported that phosphazenes are potential biodegradable molecules in vivo and in vitro, and ultimately convert to harmless fragments, especially polyphosphazenes. Also, Öztürk et al. [14] and Konar et al. [15] have stated that monophosphazene compounds are biodegradable and ultimately are converted to harmless fragments.

In our studies, we found that the lipid level of the *S. cerevisiae* decreased in triphenyl monophosphazene and tri(*o*-tolyl)monophosphazene groups (Table 6). Gunstone et al. [20] have reported that fungal lipids are affected by certain factors such as temperature, pH, carbon source, inorganic nutrients, and aeration. In addition it has been stated that the most important nutritional parameter for fungal lipid synthesis is the ratio of carbon to nitrogen. High lipid accumulation is associated with low protein synthesis and is therefore favored by high ratios of carbon:nitrogen. The highest accumulation of fats by many fungi is obtained in nitrogen deficiency media [21]. Our results showed that the triphenyl monophosphazene depressed the lipid level of *S. cerevisiae*. It was found that the monophosphazene compounds such as alkoxy and isopropyloxy decreased the lipid level of yeast cells. The decline of the lipid level in the cells may be due to raised protein synthesis. In general, lipid accumulation is more favored when nitrogen is supplied from an inorganic source, since organic nitrogen tends to stimulate growth and protein synthesis. Some fungal species are able to grow successfully on media containing vitamins and vitamin deficiencies result in a reduction of lipid content. The lipid level of *S. cerevisiae* had no effect by low vitamin E dose, but its high concentration decreased the lipid level in cell pellet. However, certain yeast cells when grown on vitamin deficient media, accumulate large amounts of triacylglycerides [23]. Öztürk et al. [14] and Konar et al. [15] found that the lipid level of *S. cerevisiae* was decreased by vitamin E. Also, Yılmaz et al. [24] found that the lipid level was decreased by vitamin E and selenium in testes tissue of lamb.

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