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Synthesis and Evaluation of New Anti-microbial Additives Based on Pyrazole and Triazole Derivatives Incorporated Physically into Polyurethane Varnish for Surface Coating and into Printing Ink Paste

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PYRAZOLE, triazole, pyramidine, phenylsulfone and their derivatives are some of the oldest and best known class of nitrogen and sulphur containing compounds. In the recent years there has been considerable interest in phenylsulfonyl, pyrazolo and phenylsulfonyl triazolo derivatives due to the presence of the important pharmacophore groups. In this particular research, certain compounds as 5-(methylthio)-6-(phenylsulfonyl)-3-(*p*-tolyldiazenyl)-pyrazolo [1,5-*a*] pyrimidine-2,7-diamine (compound II), 3-((4-metho-xyphenyl) diazenyl) -5-(methylthio) -6-(phenylsulfonyl) pyrazolo [1,5-*a*]pyrimidine-2,7-diamine (compound III) and 5-(methylthio)-6-(phenylsulfonyl) [1,2,4]triazolo[1,5-*a*] pyrimidin-7-amine (compound IV) were prepared and their structure was confirmed by spectral data and also screened for their antimicrobial activity against six different micro-organisms when physically incorporated into polyurethane varnish formula and printing ink paste. Experimental coatings were manufactured on a laboratory scale and applied by brush on glass and steel panels. Results of the biological activity indicated that polyurethane varnishes and printing ink paste containing compounds II, III and IV exhibit a very good antimicrobial effect. The physical and mechanical resistances of the polyurethane varnish formulations were also studied to evaluate any drawbacks associated with this addition. The studies revealed that the physical incorporation of compounds II, III and IV marginally enhances both physical and mechanical properties.

Keywords: Pyrazole and triazole derivatives, biocides, Anti-microbial, Polyurethane coating and Printing ink paste.

Polyurethane, polyester and polyesteramide are susceptible to microbial attack, when they are exposed to atmosphere, or used as an adhesive, or as a coating material. Generally microorganisms have been found to cause disbanding and blistering of coatings under various service conditions [1–3] . Marine biofouling is a natural phenomenon representing one of the greatest problems in marine technology and navigation, since the accumulation of organisms such as barnacles, tube worms and algae on the submerged surfaces of the vessels results in important speed reduction and considerably higher fuel consumption. To circumvent these problems, antifouling paints, *i.e.* paint formulations traditionally containing biocidal species, are used to protect the submerged surfaces from marine biofouling [4] . Till the end of 1990s, the most effective antifouling paints were based on organotin compounds, mostly tributyltin compounds (TBT-based paints). TBT and its derivatives were found to be harmful molecules to marine ecosystems by Alzieu [5] and so it is completely prohibited from January 1st 2008 [6–8] . This restriction has promoted further research into new eco-friendly marine paints. One of the methods to overcome such a problem is to develop polymers having biocidal activities [9] . A large number of naturally occurring compounds contain heterocyclic rings as an important part of their structure such as coumarin (IUPAC name: *2H*-Chromen-2-one) compounds and its derivatives are used as medicines [10] . Coumarin compounds and their derivatives form a group of more than 40 drugs, which are widely used in medicine as anticoagulant, hypertensive, antiarrhythmic, immunomodulant agents [11] and possess remarkable activities against bacteria [12] and fungi [13] . Furthermore, pyrimidine derivatives having various substituted thiazole rings at carbon-3 exhibit promising biological activities [14] . Heterocyclic compounds based on sulfer have attracted continuing interest because of their varied biological activities [15] , which have found applications in the treatment of microbial infections [16–17] . Thiazole and pyrozol are parent materials for various chemical compounds including sulfur drugs, biocides, fungicides, dyes, and chemical reaction accelerators. In addition, 2-amino thiazole derivatives are reported to exhibit significant biological activities and are widely used as pharmaceuticals [18] . On the basis of all of this evidence, this study reports the synthesis, characterization and antimicrobial activities of new structure hybrids incorporating phenylsulfone and pyrazol (or:triazole) ring system. This combination was anticipated to have an influence on the biological activities. The heterocyclic compound based on 3,3-bis(methylethio)-2-(phenylsulfonyl)acrylonitrile, as 5-(methylthio)-6-(phenylsulfonyl)-3-(*p*-tolyldiazenyl)pyrazolo[1,5-*a*]pyrimidine-2,7-diamine (compound II), 3-((4-methoxyphenyl)diazenyl)-5-(methylthio)-6-(phenyl-sulfonyl) pyrazolo[1,5-*a*]-pyrimidine-2,7-diamine (compound III) and 5-(methylthio)-6-(phenylsulfonyl)[1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine (compound IV) were physically added to the polyurethane varnish and printing ink paste, to make it antimicrobial. The biological activity test was used to assess the biological activity of the additive. The physical and mechanical resistances were also studied to evaluate any drawbacks associated with the additive.

Experimental

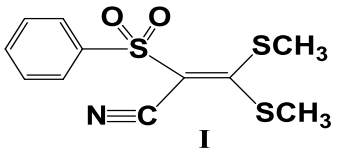
Materials

All the chemicals used during the project were sourced locally or internationally, and are of high purity grade.

Methods and Techniques

New anti-microbial additive based on pyrazole and triazole derivatives (compound II, III & IV) were prepared as shown in Scheme 1 and as discussed below.

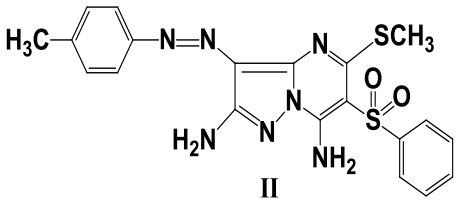
Synthesis of 3,3-bis(methylthio)-2-(phenylsulfonyl)acrylonitrile as starting material (compound I)



I

A solution of 2-(phenylsulfonyl)acetonitrile (0.01mol) and sodium ethoxide (0.46 g, 0.02 mol) in 20 ml absolute ethanol were refluxed for 20 min. After cooling, carbon disulfide (0.80 ml, 0.01 mol) was added. The reaction mixture was stirred at room temperature for 30 min. Once the mixture had cooled, methyl iodide (2.80 ml, 0.02 mol) was added and stirred at room temperature for 2 hr. The mixture was poured on ice. The precipitated product was filtered off and recrystallized from ethanol to give 3,3-bis(methylthio)-2-(phenylsulfonyl)acrylonitrile.

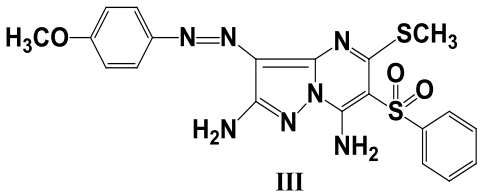
Synthesis of 5-(methylthio)-6-(phenylsulfonyl)-3-(p-tolyldiazenyl)pyrazolo-[1,5-a]pyrimidine-2,7-diamine (compound II)



II

A mixture of 10 mmol of both compound (I) and 4-(*p*-tolyldiazenyl)-1*H*-pyrazole-3,5-diamine, in ethanol (25 ml), together with a few drops of piperidine, was refluxed for 3 hr. The solid product was filtered off, washed with ethanol and recrystallized from ethanol/DMF to afford the title compound.

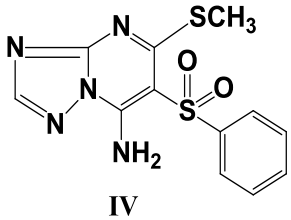
Synthesis of 3-((4-methoxyphenyl)diazenyl)-5-(methylthio)-6-(phenylsulfonyl) pyrazolo[1,5-a]pyrimidine-2,7-diamine (compound III)



III

A mixture of 10 mmol of both compound (I) and 4-((4-methoxyphenyl) diazenyl)-1*H*-pyrazole-3,5-diamine (10 mmol) in ethanol (25 ml), together with a few drops of piperidine, was refluxed for 12 hr. Following reflux completion, the solvent was evaporated and the crude product was then taken in ethanol, and then collected by filtration, dried and recrystallized from ethanol/DMF to afford the title compound.

Synthesis of 5-(methylthio)-6-(phenylsulfonyl)[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (compound IV)

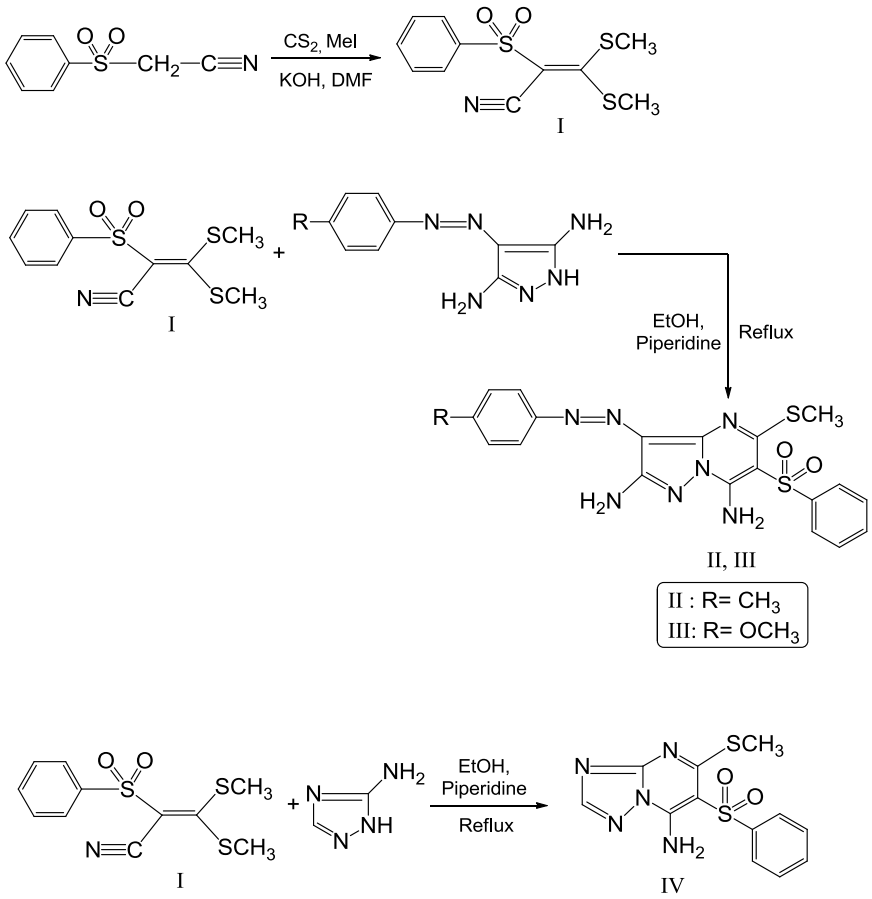


IV

An equimolar amount (10 mmol) of compound (I) and 5-amino-l,2,4-triazole in presence of pyridine (25 ml) was refluxed for 10 hr, then left to cool. The solvent was evaporated and the residual solid was taken in ethanol then collected by filtration, dried and finally recrystallized from DMF/H_2O to afford the title compound.

Characterization studies

Melting points were determined with a Stuart Scientific Co. Ltd., apparatus. Elemental analyses were performed on a Perkin-Elmer 240 micoanalyser at the Micro analytical Center of Cairo University. The IR spectra (KBr technique) were recorded on a FTIR 5300 spectrometer (ν, cm^{-1}). ^{1}HNMR spectra (DMSO-d_6) were recorded on a Varian Gemini 300 MHz spectrometer and chemical shifts are expressed in δ ppm units, using TMS as an internal standard.



Scheme1. Synthesis of new anti-microbial additives based on pyrazole and triazole derivatives (compounds I, II, III & IV) .

Preparation of antimicrobial (biocidal) coating

The prepared new additives based on pyrazole and triazole derivatives (compound II, III & IV) were incorporated physically into commercial reference polyurethane varnish and printing ink paste with ratio's of 0.5 and 1.0 weight percent respectively. The composition of the used polyurethane varnish and the printing ink paste used in the study is tabulated in Tables 1&2. The samples of different molar ratio were then applied onto both glass and steel panels by means of a brush. All efforts were made to maintain a uniform film thickness of 50 +/- 5µm for evaluating the physical and mechanical properties.

TABLE 1 . Composition of the polyurethane varnish studied.

Component	**wt %**
Refined sunflower oil	33.42
Glycerol	0.039
Litharge (lead oxide catalyst)	0.03
Pentaerythritol	4.61
Turpentine	47.33
Barium octoate drier	0.26
Toluene diisocyanate	11.37
Mixed drier	2.11
UV absorber	0.26
Anti skinning agent	0.32
Biocide additive (compounds II, III & IV)	0.5,1.0

Properties: viscosity: G-I (gardner), color: 3 (gardner), solid content: 53±2%.

TABLE 2 . A) Printing ink paste formulation 1.

Component	**wt %**
Binder	30
TiO_2	20
$CaCO_3$	30
Dispersing agent	0.5
Antifoam	1,0
Biocide additive (compounds II, III & IV)	0.5
H_2O	balance

TABLE 2 . B) Printing ink paste formulation 2.

Component	**wt %**
Binder	14.5
Pigment (printofix Black R-G clariant, India)	3
Thickeners (Argoprint 165 A)	2
Biocide additive (compounds II, III & IV)	0.5
H_2O	balance

Antimicrobial screening

The anti-microbial activity of the synthesized pyrazole and triazole derivatives (compound II, III & IV) was tested against six different micro-organisms namely, i)Gram-negative bacteria (G-) [*Escherichia coli* (*E. coli*) & *Salmonella typhimurium*], ii) Gram-positive bacteria (G+) [*Micrococcus luteus* (*M. luteus*), *Staphylococcus aureus* (*St. aureus*)] and iii) against fungi [*Aspergillus flower* (*A. flower*), *candida albicans*]. Nutrient agar was used as the medium.

Paper disc diffusion method for determination of antimicrobial activity

The paper disc diffusion method is used to determine how antibiotics or compounds inhibit bacterial or bacteriostatic growth. The paper discs are soaked with a selected antibiotic or chemical and then placed on a lawn of bacteria in a petri dish. The zones of inhibition were measured around where the disc was placed to determine whether the bacterium was resistant or susceptible to the particular antibiotic or chemical chosen. The sterilized (autoclaved at 120 °C for 30 min) medium at (40-50 °C) was incubated (1 ml/100 ml of medium) with the suspension (10^5 cfu ml^{-1}) of the micro-organism (matched to McFarland barium sulphate standard) and poured into a petri dish to give a depth of 3-4 mm. The paper impregnated with the test compounds (mg/ml^{-1}) was placed on the solidified medium. The plates were pre-incubated for 1 hr at room temperature and incubated at 37 °C for 24 and 48 hr for anti-bacterial and anti-fungal activities, respectively. Cefepime (mg/disc) was used as a standard for antibacterial and anti-fungal activity, respectively.

Minimum inhibitory concentration (MIC) test for determination of anti-microbial activity

MIC was determined by the agar streak dilution method. A stock solution for each synthesized compounds, of concentration (100 mg/ml^{-1}) in dimethyl formamide, was prepared and incorporated in specified quantities of molten sterile agar (nutrient agar for anti-bacterial activity and sabouraud dextrose agar medium for anti-fungal activity). A specified quantity at temperature of (40-50 °C) containing the prepared compounds was poured into a Petri dish to give a depth of 3-4 mm and allowed to solidify. A suspension of the micro-organism was prepared to contain approximately (10^5 cfu ml^{-1}) and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37°C for 24 and 48 hr for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate.

Physical and mechanical testing of films

A range of physical and mechanical evaluations of the paint films were undertaken according to appropriate ASTM standard test methods. The color of polyurethane varnish formulations was measured using a Gardner standard color scale (ASTM D1544). The prepared steel panels (ASTM D 609-95) were used to measure the film coating thickness (ASTM D 1005-07), the adhesion 'cross hatch' test (ASTM D 3359-02) and the flexibility 'bend' test (ASTM D 522-93a). Glass plates (100x150mm) coated with the individual formulations were utilized to measure the degree of gloss at an angle of 20° (ASTM D 523-08) and to measure film hardness by means of the pencil test (ASTM D 3363-00).

Results and Discussion

In 1937 the birth of polyurethanes (PUs) occurred in Germany when Bayer laboratories explored their use as fiber-forming polymers [19]. Polyurethanes are an important and very versatile class of polymer materials with desirable properties, such as high abrasion resistance, tears strength, excellent shock absorption, flexibility and elasticity [20-22] . The attractiveness of polyurethanes stems from their excellent bonding to different substrates, relatively low price and fast reaction time [23]. Polyurethane top-coats, recommended for paint systems commonly utilized for corrosion protection of steel structures, are used in highly corrosive atmospheres (C5 category) [24]. On the basis of these former studies, and their respective findings, we have selected a polyurethane vanish formulation containing the new prepared compounds to study the antimicrobial activities.

Synthesis of new anti-microbial additive based on pyrazole and triazole derivatives (compound II, III & IV)

The synthesis of a new anti-microbial additive based on pyrazole and triazole derivatives (compounds II, III & IV) was prepared in the hope that it might demonstrate enhanced antimicrobial activity properties. The chemical structure of the prepared compounds II, III & IV is represented in Scheme 1. In this series all compounds were prepared and purified as illustrated in the experimental section. The good agreement between the experimental and theoretical values of the C, H and N and spectrophotometric studies levels reveals that the methods of synthesis and purification of the products were performed successfully. Elemental analysis, reaction yield and physical properties, such as melting point and product color, were measured and listed in Table 3.

TABLE 3. Physical and elemental analysis data of the prepared compounds I- IV.

No.	M.p. ºC (Solvent)	Yield % (Color)	Mol. Formulae (M.Wt.)	Analysis: Calcd / Found			
				C%	H%	N%	S%
I	126-128 (EtOH)	85	$C_{11}H_{11}NO_2S_3$ (285.41)	46.29 46.44	3.88 3.92	4.91 4.83	33.70 33.59
II	256-258 (EtOH/DMF)	68	$C_{20}H_{19}N_7O_2S_2$ (453.54)	52.96 53.07	4.22 4.31	21.62 21.50	14.14 14.06
III	264-266 (EtOH/DMF)	66	$C_{20}H_{19}N_7O_3S_2$ (469.54)	51.16 51.22	4.08 4.14	20.88 20.80	13.66 13.59
IV	266-268 (DMF/H_2O)	80	$C_{12}H_{13}N_5O_2S_2$ (323.39)	44.57 44.66	4.05 4.13	21.66 21.59	19.83 19.73

Spectroscopy studies

Spectral analysis of 3,3-bis(methylthio)-2-(phenylsulfonyl)acrylonitrile as starting material (compound I)

- IR (KBr) ν_{max}/cm^{-1}: 2195 (C≡N), 1308, 1149 (SO_2).
- ^1H NMR (DMSO-d_6): δ 2.71 (s, 6H, $2SCH_3$), 7.67-7.98 (m, 5H, C_6H_5).
- MS (m/z): 285(M+).

Spectral analysis of 5-(methylthio)-6-(phenylsulfonyl)-3-(p-tolyldiazenyl)-pyrazolo[1,5-a]pyrimidine-2,7-diamine (compound II)

- IR (KBr) vmax/cm^{-1}: 3371, 3321 (NH_2), 1546 (C=N), 1384, 1134 (SO_2).
- ^1H NMR (DMSO-d_6): δ 2.26 (s, 3H, CH_3), 2.37 (s, 3H, SCH_3), 6.09 (br's, 2H, NH_2, D_2O-exchangable), 7.17-7.61 (m, 9H, ArH's), 10.73 (br's, 2H, NH_2, D_2O-exchangable).
- MS (m/z): 452(M+).

Spectral analysis of 3-((4-methoxyphenyl) diazenyl)-5-(methylthio)-6-(phenylsulfonyl) pyrazolo[1,5-a]pyrimidine-2,7-diamine (compound III)

- IR (KBr) vmax/cm^{-1}: 3394, 3178 (NH_2), 1496 (C=N), 1369, 1134 (SO_2).
- ^1H NMR (DMSO-d_6): δ 2.45 (s, 3H, SCH_3), 3.73 (s, 3H, OCH_3), 5.98 (br's, 2H, NH_2, D_2O-exchangable), 6.93-8.01 (m, 9H, ArH's), 10.65 (br's, 2H, NH_2, D_2O-exchangable).
- MS (m/z): 469(M+).

Spectral analysis of 5-(methylthio)-6-(phenylsulfonyl)[1,2,4]triazolo[1,5-a]pyrimidin-7-amine (compound IV)

- IR (KBr) vmax/cm^{-1}: 3364, 3250 (NH_2), 1585 (C=N), 1338, 1126 (SO_2).
- ^1H NMR (DMSO-d_6): δ 2.31 (s, 3H, SCH_3), 3.67 (s, 2H, NH_2, D_2O-exchangable), 7.58-7.88 (m, 6H, ArH's and 1H, triazole-3-CH).
- MS (m/z): 323(M+).

Evaluation of new anti-microbial additives based on pyrazole and triazole derivatives (compound II, III & IV) as biocide additives incorporated into a polyurethane varnish and printing ink paste

Biocide additives have been used for prolonging the life of surface coatings. They prevent, or slow down, the growth of organisms on the surface coating. Without biocide additives, the biological species start to adhere to the coating surface, which can cause disbonding and blistering of coatings under various service conditions. Biocide additives fall into two main categories; those used for wet state (in-can) protection to prevent bacteria and fungi from spoiling paint during storage until it can be applied, and those used to prevent fungi and algae from growing on the applied paint film. Phenylsulfone and pyrazol (or:triazole) derivatives are some of the oldest and best known class of nitrogen and sulphur containing compounds. In the recent years there has been considerable interest in the phenylsulfone and pyrazol (or:triazole) based family of materials due to the range of antibacterial and

chemical stabilities associated with them. In addition, these materials are capable of imparting antimicrobial activity properties when incorporated into polymers and their composites. The results obtained from the antimicrobial activity are shown in Tables 4&5 and Fig. 1&2. The antimicrobial activity of the blank and blended polyurethane varnish formulations and printing ink paste was evaluated by testing it against six different micro-organisms, including Gram-negative bacteria (G-), Gram-positive (G+) bacteria and fungi. It is clearly seen from the results that compounds II, III & IV show i) moderate antimicrobial activity against Gram-negative bacteria (G-) [*Escherichia coli*], higher sensitivity than salmonella typhimurium, ii) high antimicrobial activity against Gram-positive bacteria (G+) highly effective against Micrococcus luteus and *Staphylococcus aureus*], iii) mild antimicrobial activity against fungi [*Aspergillus flower* and showed the highest sensitivity for *Candida albicans*]. It can be observed that the antimicrobial activity against the target micro-organisms increases with the increase in the biocide additive content and it gives better results with polyurethane than printing ink paste. This is due to the incorporation of compound II, III & IV into the polyurethane varnish formulations and printing ink paste. This enhancement may be attributed to a number of key factors. Firstly, the introduction of a heterocyclic compound based on sulfone moiety which possesses remarkable activities against bacteria and fungi. Secondly, the introduction of pyrazole (or:triazole) and pyrimidine ring, which is also a familiar group of heterocyclic compounds possessing remarkable activities against bacteria. Thirdly, the introduction of free amino groups' derivative which is reported to exhibit significant biological activities.

TABLE 4. Anti-microbial activity of polyurethane varnish incorporated new pyrazole and triazole derivatives (compound II , III & IV) as a biocide additive.

Microorganisms	Blank	Compound II %		Compound III %		Compound IV %	
		0.5	1.0	0.5	1.0	0.5	1.0
Micrococcus luteus (ATCC 9341)	-Ve	++Ve	++++Ve	+++Ve	++++Ve	++Ve	++++Ve
Staphylococcus aureus (NCTC 7447)	-Ve	++Ve	+++Ve	++Ve	++++Ve	++Ve	++++Ve
Escherichia coli	-Ve	+Ve	++Ve	++Ve	+++Ve	+Ve	++Ve
Salmonella typhimurium	-Ve	++Ve	+++Ve	++Ve	++++Ve	++Ve	++++Ve
Candida albicans (IMRU 3669)	-Ve	++Ve	+++Ve	++Ve	++++Ve	++Ve	+++Ve
Aspergillus flavus	-Ve	+Ve	++Ve	++Ve	+++Ve	+Ve	+++Ve

Where, Inactive = -Ve,
Mildly active: Inhibition values = 0.1–0.6 cm beyond control = +.
Moderately active: Inhibition values =0.65–1.0 cm beyond control =++.
Highly active: Inhibition values = 1.1–1.5 cm beyond control =+++.
Very highly active: Inhibition values = 1.6–2.00 cm beyond control =++++.

TABLE 5. Anti-microbial activity of printing ink paste incorporated new pyrazole and triazole derivatives (compound II , III & IV) as a biocide additive.

Microorganisms	Blank	Compound II %		Compound III %		Compound IV %	
		Paste 1	Paste 2	Paste 1	Paste 2	Paste 1	Paste 2
Micrococcus luteus (ATCC 9341)	-Ve	++Ve	++++Ve	++Ve	++++Ve	++Ve	++++Ve
Staphylococcus aureus (NCTC 7447)	-Ve	++Ve	+++Ve	++Ve	++++Ve	++Ve	+++Ve
Escherichia coli	-Ve	+Ve	++Ve	++Ve	++Ve	+Ve	++Ve
Salmonella typhimurium	-Ve	++Ve	+++Ve	++Ve	+++Ve	++Ve	+++Ve
Candida albicans (IMRU 3669)	-Ve	+Ve	+++Ve	+Ve	+++Ve	++Ve	++Ve
Aspergillus flavus	-Ve	++Ve	++Ve	++Ve	++Ve	+Ve	++Ve

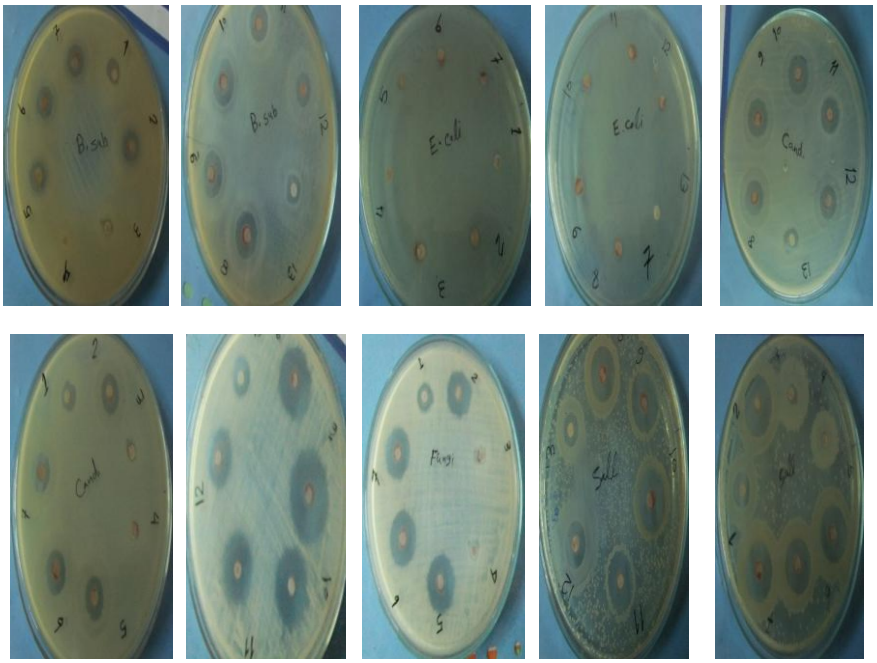


Fig.1. Anti-microbial activity of polyurethane varnish incorporated new pyrazole and triazole derivatives (compound II , III & IV) as a biocide additive.

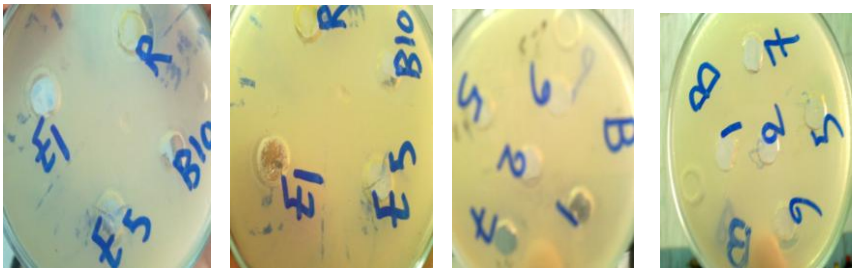


Fig. 2. Anti-microbial activity of printing ink paste incorporated new pyrazole and triazole derivatives (compound II , III & IV) as a biocide additive.

Evaluation of the physical and mechanical properties of polyurethane varnish formulations containing the new antimicrobial as biocide additive

The effects of adding an anti-microbial additive to the polyurethane varnish, in respect of the physical and mechanical properties, were evaluated as per the standard test methods. This was done to ascertain any negative aspects that might arise due to the presence of the additives. The color, gloss, scratch hardness, adhesion and flexibility were all measured. The resulting data is shown in Table 6. All modified and unmodified polyurethane varnish compositions showed very clear transparent and homogenous appearance following the addition of the anti-microbial additives.

TABLE 6. Physical and mechanical characteristics of polyurethane varnish incorporated new pyrazole and triazole derivatives (compound II, III & IV) as a biocide additive.

Characteristics	Blank	Compound II %		Compound III %		Compound IV %	
		0.5	1.0	0.5	1.0	0.5	1.0
Color "Gardner"	14	>18	>18	>18	>18	>18	>18
Gloss at 20°C	83	92	94	85	85	87	90
Scratch hardness (Kg)	>1.5	>1.5	>1.5	>2	>1.5	>2	>2
Adhesion	4B	5B	5B	5B	5B	5B	5B
Flexibility	Pass	Pass	Pass	Pass	Pass	Pass	Pass

(a) Color

Color was measured using the Gardner standard colors which consists of 18 colors numbered from 1 to 18. The method determines the color by comparison with standards of definite color compositions. It could be seen that the antimicrobial additive actually increased the color levels. This is obviously a negative result which may be attributed to the introduction of sulphur and nitrogenous base into polyurethane varnish formulations.

b) Gloss

Gloss was measured using a standard glossmeter (Sheen UK). On measuring the films at 20° angle, it was observed that the flame retardant additives increased the gloss. This is a positive result which may be attributed to the introduction of aromatic rings within the structure of the additives.

c) Scratch hardness test

It was determined by using a scratch hardness tester (Sheen UK). The scratch hardness varies from 1000 - 2000 g. It is clear from the data that as we increase the level of the additives, there is an increase in the scratch hardness of the film.

d) Cross-hatch adhesion test

It was measured by using a crosscut adhesion tester (Sheen U.K.). In this test method a lattice with six cuts in each direction is made in the film to the substrate (space the cuts 1 mm), pressure-sensitive tape is applied over the lattice and then removed. All the coating films demonstrated good cross-hatch adhesion.

e) Flexibility (bend) test

Flexibility was determined by using a ¼ inch Mandrel bend tester (Sheen U.K.), in such a way that the surface of the panel was directed outside. Films of all the coating compositions passed the ¼ inch mandrel bend test. The varnish was considered satisfactory if no marks for cracking or dislodging are observed after bending. Based on this qualitative measurement, it can be said that all the films had reasonably good flexibility.

Conclusion

Biocide additives have been used to prevent or slow down the growth of organism on the surface coating. They are essential for surface coating, as without biocide additives the biological species start to adhere to the coating surface, which can lead to disbanding and blistering of coatings under various conditions. In this study a new antimicrobial additive, 5-(methylthio)-6-(phenylsulfonyl) -3-(*p*-tolyldiazenyl) pyrazolo [1,5-*a*]pyrimidine-2,7- diamine (compound II), 3-((4-methoxyphenyl) diazen-yl) -5- (methylthio) -6- (phenylsulfonyl) pyrazolo [1,5-*a*] pyrimidine-2,7-diamine (compound III) and 5-(methylthio) -6- (phenylsulfonyl) [1,2,4] triazolo [1,5-*a*] pyrimidin-7-amine (compound IV), was physically applied to polyurethane varnish and printing ink paste as a biocide additive. Its antimicrobial activity was tested against six different micro-organisms. The antimicrobial activity against the target microorganisms increases with an increase in the level of the biocide additives. This is due to the incorporation of compound II, III & IV into the polyurethane varnish formulations and printing ink paste. This enhancement may be attributed to a number of key factors. Firstly, the introduction of a heterocyclic compound based on sulfone moiety which possesses remarkable activities against bacteria and fungi. Secondly, the

introduction of pyrazole (or:triazole) and pyrimidine ring, which is also a familiar group of heterocyclic compounds possessing remarkable activities against bacteria. Thirdly, the introduction of free amino groups' derivative which is reported to exhibit significant biological activities. The physical and mechanical properties were examined to ascertain any drawbacks. The incorporation of an anti-microbial additive into a polyurethane varnish results in a marginal enhancement of both physical and mechanical properties.

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تشييد بعض مشتقات البيرازولو و الترايازولو, واختبار فاعلية هذه المشتقات كإضافات مضادة للميكروبات عند استخدامها في تركيبات طلائية من ورنيشات البولى يوريثان وفي تركيبات من أحبار الطباعة

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تعد المركبات الكيميائية المحتوية على حلقة البيرازول أو الترايازول أو البيريميدين أو تلك المحتوية على مجموعة السلفون جزءاً هاما وأساسياً من المركبات الكيميائية العضوية. فأهميتها ترتبط بتطبيقاتها المختلفة سواء في المجالات الطبية الواسعة أو المجالات الصناعية. ولذلك فقد كان الهدف من هذا البحث هو تحضير مشتقات البيريميدينات المندمجة بحلقة البيرازول أو الترايازول والمحتوية على مجموعة السلفون.

فقد أمكن تحضير مشتقات من هذا النوع بتفاعل مشتق ثنائي ثيوأسيتال مع مشتقات 5,3- ثنائي أمينو-4-أريل آزو بيرازول ومع 5-امينو-4,2,1-ترايازول ليعطي مشتقات البيرازولوا[*a*-5,1]بيريميدين (II , III) و مشتق الترايازولو[*a*-5,1]بيريميدين (IV). وقد تم إثبات التراكيب البنائية للمركبات السابقة بالوسائل الطيفية المختلفة مثل طيف الأشعة تحت الحمراء وطيف الكتلة وكذلك طيف الرنين النووي المغناطيسي لعنصر الهيدروجين.

كما تم دراسة وتقييم تأثير هذه المشتقات كإضافات محسنة للخواص الفيزيائية والكيميائية وأيضا البيولوجية عند استخدامها في تركيبات طلائية من راتنجات البولي يوريثان أو تركيبات أحبار مختلفة وذلك بغرض إمكانية الاستفادة منها في التطبيق الصناعي.

حيث تضمنت الدراسة اختبار الفاعلية البيولوجية لتركيبات طلائية من راتنجات البولي يوريثان كحوامل لطلاء السطوح الخشبية بإضافة المركبات (II , III & IV) ودراسة مقاومتها للنشاط الميكروبي ضد البكتيريا سالبة الجرام أو موجبة الجرام وكذلك ضد الفطريات وتقييم مدى تأثيرها على هذه الميكروبات. وقد دلت النتائج على التغير الملموس للخواص البيولوجية للراتنجات المطورة وقدرة هذه المركبات على مقاومة الميكروبات. وقد تم أيضاً دراسة تأثير تلك المركبات المضافة على الخواص الفيزيائية للراتنجات المطورة مثل قياس اللزوجة واللمعان والمرونة ومقاومة الخدش والتقشر. وقد أثبتت النتائج مدى التحسن الملحوظ في زيادة درجة اللزوجة واللمعان والمرونة علاوة على زيادة مقاومة الخدش والتقشر.

وامتدت الدراسة لتشمل أيضا مدى تأثير المركبات (II , III & IV) عند استخدامها في مكونات الأحبار الطباعية, ودراسة مقاومتها للنشاط الميكروبي والفطري, وقد أعطت نتائج جيدة.