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Biological Evaluation of Some Azole Derivatives in Cooling Fluids (Lubricant Oils)

Firas Abdullah Hassan and Khalid Waleed Younus
Department of Chemistry, College of Science, University of Al-Nahrain, Al-Jadryriah, Baghdad, Iraq

Abstract: In the present study, a series of five members heterocyclic where used as antimicrobial against the pseudomonas bacteria which have been found in the cooling fluids of industrial purposes. The results showed that the compounds 3 and 5 are relatively higher percentage in killing bacteria than other compounds used in this research indicating that from measuring pH, turbidity and culture media to inhibit the pseudomonas bacteria. After measuring the microorganisms growth of cooling fluids in 3 different periods (1 day, 1 and 2 weeks) by using pH and turbidity measurements enhanced with media culture method, the 2 weeks sample had been taken which showed highest growth of microorganisms meanwhile these azoles derivatives have been used as antimicrobial against the microorganisms which have been found in the cooling fluid of industrial purposes by measuring pH and turbidity enhanced with media culture the results showed that the compound 5 had the best biological activity then compounds 1 and 3 are relatively higher active in killing bacteria from the compounds 2 and 4. That azoles derivative is biologically active as antimicrobial agents in cooling fluids as derivatives of azoles have used as follows: [5- (2,4-dinitrobenzamide)-2-hydrazine]-thiadiazole, 2-mercapto-1, 3, 4-triazole-2, 4-dinitrobenzamide), Dithiophen oxadiazole, Thiophene/furan oxadiazole and Phenyl/pyridine oxadiazole.

Key words: Azoles, cooling fluids, pH, turbidity, culture media, microorganism

INTRODUCTION

Azole fungicides show a broad antifungal activity and are used either to prevent fungal infections or to cure an infection. Therefore, they are important tools in integrated agricultural production. According to their chemical structure, azole compounds are classified into triazoles and imidazole, however their antifungal activity is due to the same molecular mechanism. The cell membrane assembly of fungi and yeast is disturbed by blocking the synthesis of the essential membrane component ergosterol. This fundamental biochemical mechanisim is the basis for the use of azoles fungicides in agriculture, in humane and veterinary antimycotic therapies.

The enzyme involved is sterol 14 α-demethylase which is found in several phyla, in mammals, it converts lanosterol into the Meiosis-Activating Sterols (MAS). Azole compounds play a key role as antifungal in agriculture and in human mycosis and as non-steroidal antieserogens in the treatment of the estrogen-responsive breast tumor in postmenopausal women. Cooling fluids are solutions or emulsions often prepared from mineral oils or artificial oils which are used in the mechanical works like cutting and smoothing, the first use of these kind of oils goes back to 200 years ago, those oils were used alone at that time by putting it with a brush on the cutting tool (Xin-Ping *et al*., 1999). Cooling fluids has two main jobs, these are lubricating and cooling. Lubricating is the main operation in this field in which the friction of the cutting tool with the metal which is used to cut would generate a high heat which is known as the external friction which considered to be a percentage of 1/3 from the total generated heat and there is another heat which results also from the resistance of the cutting metal part and that active under the effect of the cutting tool, this called the internal friction and the heat yield from this is 2/3%. So, the cooling fluids make to reduce from these yields heats by facilitate the slipping of the cutting tool. The second advantage is the cooling by reducing the yield heat in the metal piece (Hussain *et al*., 2008). The rise in the temperature of the metal piece will reduce the work efficiency and accuracy.

So, the high temperature will lower the working life to the cutting tool. The purity of water is the most important factor that controls the success or the fail of the cooling fluid.

The cause of deterioration for cooling fluid is the growth of many kinds of micro organisms such as; bacteria, fungi, yeast and other micro-organisms, the other cause is mixing the cooling fluids with the lubricant oils and the metal particles in which one could see the growth which effects on the oiling efficiency and makes the deterioration. Bacteria and fungi are found in the cooling fluid by the causes of (CHMR, 1992):

Corresponding Author: Firas Abdullah Hassan, Department of Chemistry, College of Science, University of Al-Nahrain, Al-Jadryriah, Baghdad, Iraq

- Finding the bacteria and the other microorganisms naturally in the useful water that used in synthesis of cooling fluids
- Deterioration of cooling fluids by microorganisms that comes from workers hands or their saliva
- Deterioration the fluids from the air of the factory's laboratory

The changes that take place on the cooling fluids because of microorganism (Howard, 1989):

- Changing the pH as well as the bad smells because of the formation of H_2S gas
- Decreasing in the cooling fluids efficiency as well as the additives specially the anti corrosion compounds
- The emulsion will be separated
- At these conditions, the microorganisms will secret organic acids which help to increase the corrosion
- The gases that result from the deterioration, the deterioration also hurts the workers by a respiration tract infection and the skin infection
- Decreasing in the quality of the yields metal as well as decreasing in the age of the cutting tool

The primary microbial culprits in the actual chemical deterioration of fluids both due to their proclivity and number of organisms belonging to the genus Pseudomonas. The most commonly encountered species is *Pseudomonas aeruginosa.* This group has a reputation of being difficult to kill and having the broadest appetite and the least nutritional need among other groups of microorganisms extant (Loida *et al*., 1989; Jaume *et al*., 2005).

MATERIALS AND METHODS

Experimental part

Equipment and apparatus: The equipment and apparatus used in this research are shown in Table 1.

Chemical and biological materials: The chemical and biological materials used in this reserach are shown in Table 2. The compounds that used in this research synthesized according to Xin-Ping *et al*. (1999) (Fig. 1). These compounds that used are:

- [5- (2, 4-dinitrobenzamide)-2-hydrazine]-thiadiazole
- 2-mercapto-1, 3, 4-triazole-2, 4-dinitrobenzamide
- Dithiophen oxadiazole
- Thiophene/furan oxadiazole
- Phenyl/pyridine oxadiazole

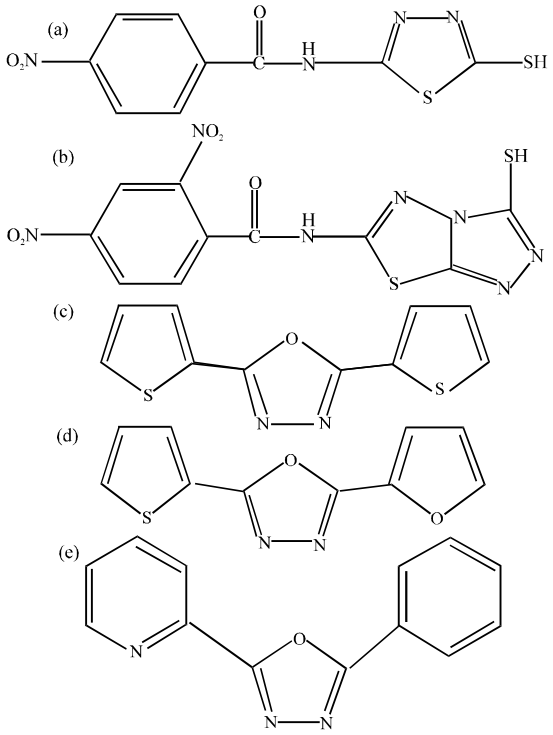

Fig. 1: Compounds used in this research, a) [5- (2, 4-dinitrobenzamide)-2-hydrazine]-thiadiazole; b) 2-mercapto-1, 3, 4-triazole-2, 4-dinitrobenzamide; c) Dithiophen oxadiazole; d) Thiophene/furan oxadiazole and e) Phenyl/pyridine oxadiazole

Table 1: The equipment and apparatus were used throughout previous study

Apparatus	Company
Sterilizer autoclave	Tomy (Japan)
Incubator	Gallen Kamp (England)
Shaker incubator	GFI (England)
Refrigeration	Concored (Turkey)
Hotplate with magnetic stirrer	Scientific (England)
Oven	Sanyo (Japan)
pH-meter	Mettler-toledo (Switzerland)
Turbidity meter	Stuart scientific (England)
Micropipettes	Volac (England)
Melting point instrument	Sanyo (Japan)

Table 2: The chemicals and biological materials used in this research

Chemical formula	State	Company
KH_2PO_4	Solid	BDH-England
K_2HPO_4	Solid	BDH-England
$MgSO_4.7H_2O$	Solid	BDH-England
$Co(NO_3)_2.6H_2O$	Solid	BDH-England
$CuSO_4.5H_2O$	Solid	BDH-England
$MnSO_4.4H_2O$	Solid	BDH-England
$FeSO_4.7H_2O$	Solid	BDH-England
NH_4Cl	Solid	Solid FluKa-Switzerland
$NiSO_4.7H_2O$	Solid	Solid FluKa-Switzerland
H_3BO_4 (Boric acid)	Solid	Solid FluKa-Switzerland
$ZnSO_4.7H_2O$	Solid	Solid FluKa-Switzerland
$CaNO_3.4H_2O$	Solid	Solid FluKa-Switzerland
Nutrient agar	Solid	Solid FluKa-Switzerland

Measuring the biological activity

Nutrient agar: Nutrient agar was prepared as recommended by manufacturing company and sterilized by autoclaving at 121AC for 15 min.

Growth of bacteria strain: Mineral salt medium was used to grow the bacteria and the aeration of liquid culture was best achieved by incubating the flask in shaker incubator at 150 rpm for 6 days at 37°C. After that 100 μL added from the emulsion samples of 1 day, 1 and 2 weeks after using to the mineral salts in conical flasks. Then these conical flasks incubated in the incubator for 6 days. After that the pH and the turbidity measured. Preparing different concentrations of the compound 1 in the mineral salts media after the growth of bacteria, the solution (0.1 g/10 mL ethanol) from compound 1 used to prepare the following concentrations that added to the conical flasks which contain 25 mL of mineral salts media with 100 μL of the emulsion sample. pH standardization has been made for each conical flask. Measuring the pH by the conical flasks before adding different concentrations of the compound 1. After addition inhibitor the conical flasks are kept in the incubator at 37°C for 48 h. The pH was measured to the 6 conical flasks after incubation period. The turbidity was measured by Turbidity meter to the 6 conical flasks. The 10 μL of control (sample emulsion) has been taken and 90 μL of sterilized normal saline was added in epindorf tube. This process has been made for each of the 6 conical flasks. The 10 μL from each epindorf tube has been taken and added to the 6 petri dishes that contain the nutrient agar and spread it. The above procedure was repeated for each inhibitor (compounds 2-5) which has been used (Unchiyama *et al*., 1988).

RESULTS AND DISCUSSION

Measuring the turbidity: The three types of samples for the fluid taken from the industrial labs in different time uses and to estimate which one of the three samples has most deteriorated a standardization process has been made to measure the turbidity and pH after adding 100 μL from each emulsion samples to the mineral salts media with incubation time, the results are shown in Fig. 2 and 3. From these data, we conclude that sample no. 3 is more deteriorated than the others (1, 2) therefore, this sample was used in measuring the biological activity of the used derivatives. The compounds (1-5) have been tested as anti-bacteria in the cooling fluid of the most deteriorated sample (2 weeks) in different concentrations then the turbidity values were measured as shown in Fig. 4. The results showed the best compound in killing bacteria that give higher turbidity is derivatives No. 5 relatively to derivative No. 3 and that is better than other derivatives (1, 2 and 4) with conc. 500 mg mL^{-1} that showed best killing percentage from other concentrations.

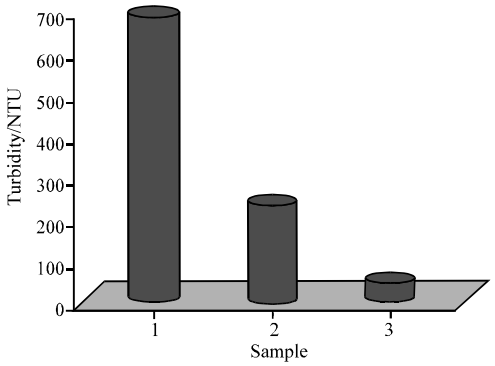

Fig. 2: The effect of the using time of the emulsion on the turbidity with the diffrent compound of azoles that used

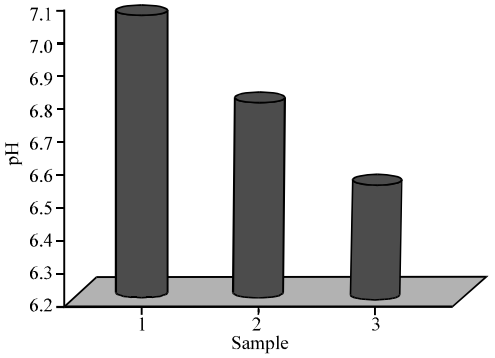

Fig. 3: The effect of the using time of the emulsion on the pH and emulsion type of it

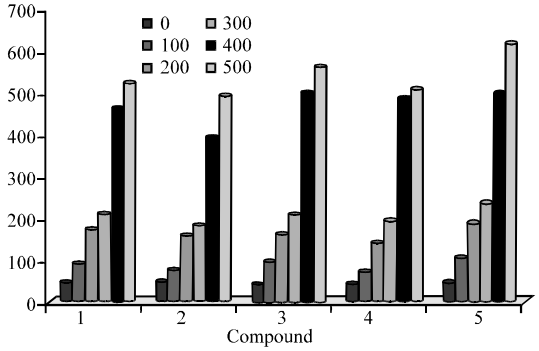

Fig. 4: The effect of the using time of the emulsion on the turbidity with the diffrent of azoles derivatives

Measuring the pH: In this research, the pH has been measured because of growth the pseudomonas bacteria in the cooling fluid that release the H_2S, so whatever the pH decreased the growth of bacteria increased (Fig. 5). These results showed the best compound in killing bacteria that give higher pH is derivatives No. 5 relatively to derivative No. 3 and that is better than other derivatives (1, 2 and 4) with conc. 500 mg mL^{-1} that showed best killing percentage from other concentrations. These results

enhanced the turbidity measurements to made compound No. 5 is the best compound in killing bacteria in cooling fluids.

Culture Method: Measuring the biological activity for the sample of 2 weeks ago after addition compound 1 as inhibitor in mineral salts media by using nutrient agar culture media. The 1st plate shows the no. of growth bacteria increased by decreasing in conc. of comp. 1 from 0-500 ppm which begin from 225-4 for diluted sample 10^{-1}. Measuring the biological activity for the sample of 2 weeks ago after addition compound 5 as inhibitor in mineral salts media by using nutrient agar culture media. The 1st plate shows the no. of growth bacteria increased by decreasing in conc. of compound 5 from 0-500 ppm which begin from 216-1 for diluted sample 10^{-1} (Table 3). The indication depend on the numbers of surviving microorganisms for each compound in different concentrations as well as the percentage of killing bacteria

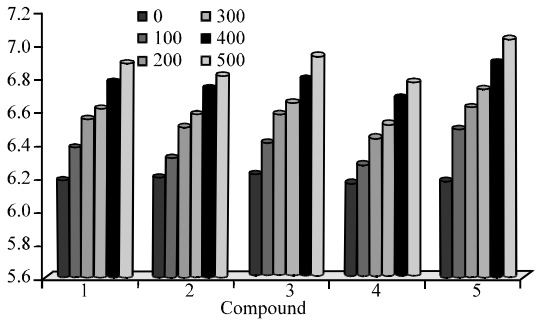

Fig. 5: The best compound in killing bacteria with different types of derivatives

Table 3: The percentage of killing for the microorganisms by thecompounds (1-5) in different concentrations

Compound no.	Control (10^{-1}) no. of surviving microorganisms	500 ppm no. of surviving microorganisms	Killing for 500 ppm (%)
1	225	4	98.34
2	230	6	97.40
3	227	3	98.78
4	246	6	97.67
5	216	1	99.64

by using each synthesized compound. So, whatever the concentration increased for each inhibitor compound, the number of the surviving bacteria decreased and the percentage of killing microorganisms increased.

CONCLUSION

In this study, researchers can notice that the best percentages of killing of the microorganisms are the compounds 5 and 3, respectively.

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