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# SYNTHESIS, BACTERICIDAL AND FUNGICIDAL ACTIVITY OF N-(4-METHOXYPHENYL) ACETAMIDE AND N-PHENYLACETAMIDE DERIVATIVES

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**Abstract.** *Introduction.* Particular attention in the cultivation of industrial and agricultural crops is paid to the fight against pathogenic fungi, which not only lead to significant yield losses, but they are also dangerous to humans. *The purpose* of this work is the synthesis of biologically active compounds in the series of aromatic dithiocarbamic acids based on N-(4-methoxyphenyl)acetamide and N-phenylacetamide and the study of their antifungal and antibacterial activities. *Results and discussion.* Biologically active aromatic sodium dithiocarbamates have been synthesized by the reaction of (N-(4-methoxyphenyl)acetamide and N-phenylacetamide) with carbon disulfide in the presence of NaOH in ethanol at 22 °C in 78 and 85% yields. Thioanhydrides have been synthesized by the acylation of sodium acetyl(4-methoxyphenyl)carbamodithioate and sodium acetyl(phenyl)carbamodithioate with acid chlorides (benzoic, 3,4-dimethoxybenzoic, 2-bromobenzoic, and 4-nitrobenzoic) in chloroform at a temperature of 22 °C. The structure of the synthesized compounds has been established on the basis of elemental analysis data, IR spectra, $^{1}$H and $^{13}$C NMR spectroscopy. *Conclusion.* Antifungal and bactericidal properties of the synthesized compounds were studied against phytopathogenic fungi and bacteria *Fusarium oxysporum* and *Pectobacterium carotovorum* bacteria. It has been found that sodium acetyl(4-methoxyphenyl)carbamodithioate exhibits high fungicidal and bactericidal activityies compared to the control and standards tetramethylthiuram disulfide (TMTD) and Fitolavin. It has been established that sodium acetyl(4-methoxyphenyl)carbamodithioate at a concentration of 0.4% has a high fungicidal activity and completely inhibits the growth of the *Fusarium oxysporum* phytopathogen. The maximum zone of inhibition (18 mm) of *Pectobacterium carotovorum* bacteria has been at a concentration of 0.4%.

**Keywords:** N-phenylacetamide, dithiocarbamates, thioanhydrides, fungicidal and bactericidal activity

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## 1. Introduction

In agricultural practice, the main control means against plant fungal diseases, which guarantees high yields, the preservation of seed material and the quality of agricultural products, is still fungicide treatment.

Dithiocarbamates receive considerable attention due to their biological activity and their key role in agriculture [1, 2]. Mancozeb, maneb, ziram, metiram, zineb, ferbam are well-known dithiocarbamate fungicides that are widely used in agricultural practice as antifungal agents for cereal and legume crops, vegetables, fruits and ornamental plants [3, 4].

Dithiocarbamate pesticides are used to treat and prevent a wide range of plant fungal diseases such as powdery mildew and septoria leaf spot, late blight, downy mildew, gray mold, scab and bacterial spot [5-11]. Their use improves seed yield and quality as a post-harvest treatment to prevent fungus or mold causing food rot during storage or transport. Dithiocarbamates are non-systemic fungicides that remain on the surface of crops after treatment and prevent fungus growth without penetrating into plant tissue.

In this regard, there is an increasing interest in the synthesis of new dithiocarbamates with a wide spectrum of action, which have a protective effect against diseases and adverse factors.

## 2. Experimental part

The progress of the reactions and the purity of the products were monitored by thin-layer chromatography on Silufol UV-254 plates with the display of spots of the compounds with iodine vapor, eluent ethanol:acetone/hexane (1/3). IR spectra were recorded on a Nicolet 5700 spectrometer in tablets with KBr. The melting points of the compounds were determined on a Hanon MP450 instrument. The $^{1}H$ and $^{13}C$ NMR spectra of the compounds were recorded on a JNM-ECA 400 spectrometer (Jeol) with the operating frequency of 400 ($^{1}H$) and 100 MHz ($^{13}C$) of the deuterated DMSO-$d_6$ and $CDCl_3$ solutions. All reagents and solvents were received from Sigma-Aldrich and used without further purification. Elemental analysis was carried out on a Rapid Micro N Cube elemental analyzer (Elementar, Germany, 2015).

*Sodium acetyl(4-methoxyphenyl)carbamodithioate (1).* A solution of 2.4 g (0.06 mol) of NaOH in 5 ml of distilled water was added to a mixture of 10 g (0.06 mol) N-(4-methoxyphenyl)acetamide in 40 ml of ethanol. Then a solution of 4.6 g (0.06 mol) carbon disulfide was added dropwise. The reaction mixture was stirred at r.t. at 22 °C for 4 h. The solvent was distilled off in a water-jet pump vacuum. The product was purified by recrystallization from acetonitrile. Yield of compound 1 was 17.27 g (78.5%), $R_f$ 0.83 (ethanol). Found, %: C 45.55; H 3.95; N 5.47; S 24.23. $C_{10}H_{10}NNaO_2S_2$. Calculated, %: C 45.61; H 3.83; N 5.32; S 24.36. NMR $^{1}H$ spectra (DMSO-$d_6$), δ, ppm: 1.95 (s, 3H, $CH_3$); 3.62 (s, 3H, $OCH_3$); 6.77 (d, 2H, $Ar_m$); 7.42 (d, 2H, $Ar_o$). NMR $^{13}C$ spectra (DMSO-$d_6$), δ, ppm: 24.2 ($CH_3$); 55.6 (O-$CH_3$); 114.3, 121.2, 132.9, 143.6, 155.6 (Ar); 168.5 (C=O); 230.6 (C=S).

*Sodium acetyl(phenyl)carbamodithioate (2).* Yield 14.6 g (85%), $R_f$ 0.80 (ethanol). Found, %: C 46.45; H 3.55; N 5.87; S 27.33. $C_9H_8NNaOS_2$. Calculated, %: C 46.34; H 3.46; N 6.00; S 27.49. IR spectra (KBr), ν, $cm^{-1}$: 1651 (C=O), 1036 (C=S), 685 (C−S). NMR $^{13}C$ spectra (DMSO-$d_6$), δ, ppm: 24.3 ($CH_3$); 119.7, 129.0, 139.2 (Ar); 169.4 (C=O); 230.6 (C=S).

*Benzoic acetyl(phenyl)carbamothioic thioanhydride (3).* A solution of 1.2 g (0.0085 mol) of benzoyl chloride was added dropwise to a solution of 2 g (0.0085 mol) sodium acetyl(phenyl)carbamodithioate in 25 ml of chloroform with stirring. The mixture was stirred at room temperature of 22 °C for two hours. The solvent was distilled off in a water-jet pump vacuum, the product was isolated by recrystallization from hexane. Yield 1.49 g (56%), $R_f$ 0.62 (acetone/hexane, 1/3), an oil. Found, %: C 60.85; H 4.27; N 4.35; S 20.41. $C_{16}H_{13}NO_2S_2$. Calculated, %: C 60.93; H 4.15; N 4.44; S 20.33. IR spectra (KBr), ν, $cm^{-1}$: 1695, 1787 (C=O), 1004 (C=S), 683 (C−S). NMR $^{13}C$ spectra ($CDCl_3$), δ, ppm: 24.5 ($CH_3$); 128.1, 128.4, 129.6, 130.6, 134.5, 134.7 (Ar); 166.6 ($CH_3C$=O); 171.8 (SC=O); 205.0 (C=S).

*3,4-Dimethoxybenzoic acetyl(phenyl)carbamothioic thioanhydride (4)* was synthesized in a similar way. Yield 2.4 g (75%), $R_f$ 0.24 (acetone/hexane, 1/3), m.p. 92 °C. Found, %: C 57.65; H 4.67; N 3.69; S 17.21. $C_{18}H_{17}NO_4S_2$. Calculated, %: C 57.58; H 4.56; N 3.73; S 17.08. IR spectra (KBr), ν, $cm^{-1}$: 1664, 1761 (C=O), 1059 (C=S), 605 (C−S). NMR $^1H$ spectra ($CDCl_3$), δ, ppm: 2.11 (s, 3H, $CH_3$); 3.89 (s, 6H, $OCH_3$); 6.91 (d, 1H); 7.03 (t, 2H); 7.22 (t, 1H); 7.50 (d, 1H); 7.57 (s, 1H), 7.74 (d, 1H). NMR $^{13}C$ spectra ($CDCl_3$), δ, ppm: 24.3 ($CH_3$); 56.1 ($OCH_3$); 110.6, 112.5, 120.3, 124.3, 125.3, 128.9, 138.2, 148.5, 149.1 (Ar); 169.6 ($CH_3C$=O); 170.7 (SC=O); 203.1 (C=S).

*2-Bromobenzoic acetyl(phenyl)carbamothioic thioanhydride (5)* was synthesized in a similar way. Yield 1.85 г (88%), $R_f$ 0.67 (acetone/hexane, 1/3), m.p. 80 °C. Found, %: C 48.65; H 3.19; N 3.69; S 16.35. $C_{16}H_{12}BrNO_2S_2$. Calculated, %: C 48.74; H 3.07; N 3.55; S 16.26. IR spectra (KBr), ν, $cm^{-1}$: 1691, 1784 (C=O), 1022 (C=S), 628 (C−S). NMR $^1H$ spectra ($CDCl_3$), δ, ppm: 2.13 (s, 3H, $CH_3$); 7.05 (t, 2H); 7.25 (t, 1H); 7.42 (t, 2H); 7.49 (d, 1H); 7.80 (d, 1H). NMR $^{13}C$ spectra ($CDCl_3$), δ, ppm: 24.5 ($CH_3$); 120.1, 124.3, 128.9, 129.4, 132.0, 138.17, 141.1 (Ar); 169.1 ($CH_3C$=O); 184.3 (SC=O); 202.7 (C=S).

*4-Nitrobenzoic acetyl(4-methoxyphenyl)carbamothioic thioanhydride (6)* was synthesized in a similar way. Yield 1.27 г (49%), $R_f$ 0.47 (acetone/hexane, 1/3), m.p. 109-110 °C. Found, %: C 52.42; H 3.79; N 7.09; S 16.35. $C_{17}H_{14}N_2O_5S_2$. Calculated, %: C 52.30; H 3.61; N 7.17; S 16.43. IR spectra (KBr), ν, $cm^{-1}$: 1687, 1765 (C=O), 1055 (C=S), 678 (C−S). NMR $^1H$ spectra (DMSO-$d_6$), δ, ppm: 1.95 (s, 3H, $CH_3$); 3.64 (s, 3H, $OCH_3$); 6.78 (d, 2H); 7.42 (d, 2H); 8.09 (d, 2H); 8.22 (d, 2H). NMR $^{13}C$ spectra (DMSO-$d_6$), δ, ppm: 24.1 ($CH_3$); 55.6 (O-$CH_3$); 114.2, 121.0, 124.0, 131.1, 133.0, 150.5, 155.5 (Ar); 166.2 ($CH_3C$=O); 168.2 (SC=O); 201.7 (C=S).

The fungicidal activity of the synthesized compounds was studied at the Kazakh Research Institute of Plant Protection and Quarantine named after Zh. Zhiembaev.

The study of fungicidal properties was carried out by the disk-diffusion method on Potato Dextrose Agar (PDA). Test object: pure culture of the fungus *Fusarium Sp*. Paper discs 10 mm in diameter were placed in Petri dishes with PDA agar medium at a distance of 2 mm from the edge of the dish and at an equal distance from each other, impregnated with a solution of the test substance. Afterwards micromycetes were sown in Petri dishes on a nutrient medium by the surface method. Incubation lasted for 7-14 days in a thermostat at a temperature of 27 °C. The presence of a inhibition zone of fungal growth was noted after 3 days. At the end of the incubation was measured the zone of fungal growth inhibition around the disk.

Determination of bactericidal properties was carried out by the well method on Potato Agar (PA). Test object: pure culture of bacteria *Pectobacterium carotovorum*, which was identified by PCR analysis. A daily culture of the test object was inoculated into Petri dishes with a nutrient medium, turbidity standard was $10^{-9}$. In the center of the Petri dish inoculated with the test object, 12 mm wells were made, into which solutions of the compounds were added. Petri dishes were placed in a thermostat at a temperature of 25-26 °C, which was optimal for bacterial growth. The presence of a zone of inhibition of bacterial growth was noted after 2 days.

Lab experience options:

1. Control (water);
2. TMTD (standard), (0.4%);
3. Phytolavin (standard), (0.5%);
4. Compound 1 (0.1%, 0.2%, 0.4%);
5. Compound 6 (0.1%, 0.2%, 0.4%).

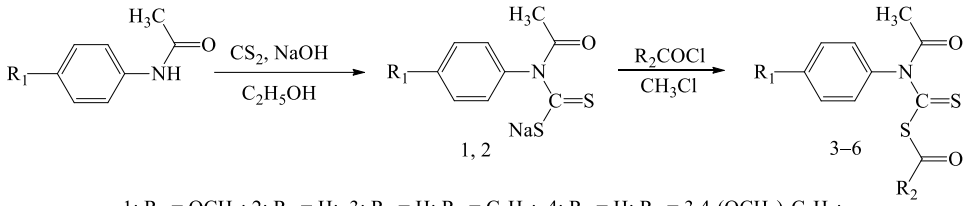
**3. Results and discussion**

The aim of the study is the targeted synthesis of new potential biologically active aromatic dithiocarbamic acids.

Sodium dithiocarbamates were obtained by reacting amines (N-(4-methoxyphenyl)acetamide and N-phenylacetamide) with carbon disulfide in the presence of NaOH in ethanol at 25 °C. Sodium acetyl(4-methoxyphenyl)carbamodithioate 1 (78.5%) and sodium acetyl(phenyl)carbamodithioate 2 (85%) were individually isolated after completion of the reactions and appropriate treatment of the reaction mixtures.

New carbonyl derivatives of thioangirides were synthesized by acylation of sodium dithiocarbamates. Acylation of dithiocarbamates 1, 2 was carried out by reacting sodium acetyl(4-methoxyphenyl)carbamodithioate 1 and sodium acetyl(phenyl)carbamodithioate 2 with acid chlorides (benzoic, 3,4-dimethoxybenzoic, 2-bromobenzoic and 4-nitrobenzoic) in chloroform medium at a temperature of 25 °C within 3 h.

As a result of isolation from reaction mixtures, thioanhydrides 3-6 were obtained individually in 49-88% yields, respectively.




1: $R_1 = OCH_3$; 2: $R_1 = H$; 3: $R_1 = H$; $R_2 = C_6H_5$; 4: $R_1 = H$; $R_2 = 3,4\text{-}(OCH_3)_2C_6H_3$;
5: $R_1 = H$; $R_2 = 2\text{-}BrC_6H_4$; 6: $R_1 = OCH_3$; $R_2 = 4\text{-}NO_2C_6H_4$;

The composition of the synthesized compounds was confirmed by elemental analysis, thin layer chromatography and physicochemical characteristics. The structure of the compounds 3-6 was established based on the analysis of IR and $^1H$, $^{13}C$ NMR spectroscopy data. The characteristic signals of protons and carbon atoms in the synthesized molecules 1-6 are presented in the experimental part.

The synthesized compounds 1 and 6 were tested for antibacterial activity against the phytopathogen *Fusarium oxysporum* and bacteria *Pectobacterium carotovorum* (Table 1, Figure 1).

**Table 1** – Fungicidal and bactericidal properties of compounds 1 and 6

| Preparation | Concentration, % | Suppression zone, mm *Fusarium Sp*. | Suppression zone, mm *P. carotovorum* |
|---|---|---|---|
| Control (Water) | | - | - |
| TMTD (Standard) | 0.4 | 5 | - |
| Phytolavin (Standard) | 0.5 | - | 4 |
| Compound 1 | 0.1 | 10 | 12 |
| | 0.2 | 17 | 15 |
| | 0.4 | clean | 18 |
| Compound 6 | 0.1 | - | 3 |
| | 0.2 | - | 3 |
| | 0.4 | - | 3 |
| Note – «-» - No zone of suppression of fungal and bacterial infection. | | | |

Analysis of the test results shows that compound 1 exhibits the highest fungicidal and bactericidal activity compared to the control and standards TMTD and Phytolavin.

The highest degree of antimicrobial activity was shown by compound 1 at the concentration of 0.4% against *Fusarium Sp*. completely inhibited the growth of the phytopathogen and *P. carotovorum* the zone of inhibition was 18 mm.

It was found that sodium dithiocarbamate 1 has a high antimicrobial activity against *Fusarium Sp*. and *Pectobacterium carotovorum*. Whereas dithiocarbamic acid thioanhydride 6 showed no activity.

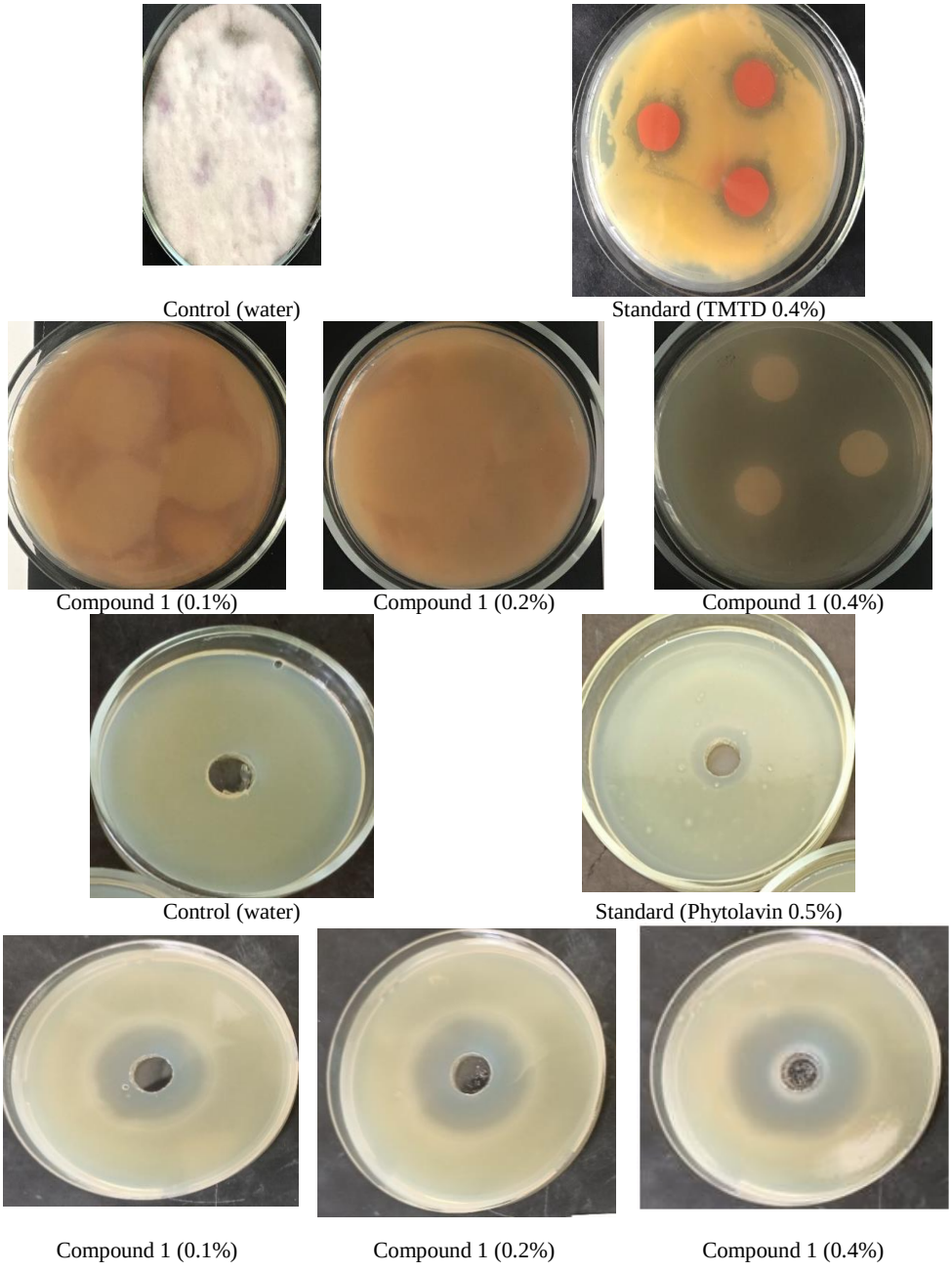



**Figure 1** – Fungicidal and bactericidal properties of compound 1.

## 4. Conclusion

As a result of this research, new thioanhydrides of aromatic dithiocarbamates based on N-(4-methoxyphenyl)acetamide and N-phenylacetamide were

synthesized. The structure of the compounds was established based on the analysis of $^{1}H$ and $^{13}C$ NMR spectroscopy data. It was established that sodium acetyl(4-methoxyphenyl)carbamodithioate has a high antibacterial activity compared to the well-known preparations TMTD and Phytolavin.

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**Conflict of Interests:** All authors declare that they have no conflict of interest.

## СИНТЕЗ, БАКТЕРИЦИДНАЯ И ФУНГИЦИДНАЯ АКТИВНОСТЬ ПРОИЗВОДНЫХ N-(4-МЕТОКСИФЕНИЛ)АЦЕТАМИДА И N-ФЕНИЛАЦЕТАМИДА

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**Резюме.** *Введение.* Особое внимание при выращивании технических и сельскохозяйственных культур уделяется борьбе с патогенными грибами, которые не только приводят к значительным потерям урожайности, но и опасны для человека. *Целью данной работы* является синтез биологически активных веществ в ряду ароматических дитиокарбаминовых кислот на основе N-(4-метоксифенил)ацетамида и N-фенилацетамида, и изучение их противогрибковой и антибактериальной активности. *Результаты и обсуждение.* Биологически активные ароматические дитиокарбаматы натрия с выходами 78 и 85% синтезированы взаимодействием аминов (N-(4-метоксифенил)ацетамида и N-phenylacetamide) с сероуглеродом в присутствии гидроксида натрия в этиловом спирте при температуре 22 ºС. Тиоангидриды синтезированы ацилированием ацетил(4-метоксифенил)карбамодитиоата натрия и ацетил(фенил)карбамодитиоата натрия хлорангидридами (бензойный, 3,4-диметоксибензойный, 2-бромбензойный и 4-нитробензойный) в среде хлороформа при температуре 22 ºС. Структура синтезированных соединений установлена на основании данных элементного анализа, ИК-спектров и спектроскопии ЯМР $^{1}H$ и $^{13}C$. *Заключение.* Изучены противогрибковые и бактерицидные свойства синтезированных соединений в отношении фитопатогенных грибов и бактерий *Fusarium oxysporum* и *Pectobacterium carotovorum*. Выявлено, что ацетил(4-метоксифенил)карбамодитиоат натрия проявляет высокую фунгицидную и бактерицидную активность по сравнению с контролем и эталонами тетраметилтиурамдисульфид (ТМТД) и Фитолавин. Установлено, что ацетил(4-метоксифенил)карбамодитиоат натрия в концентрации 0.4% обладает высокой фунгицидной активностью и полностью ингибурует рост фитопатогена Fusarium *oxysporum*. Максимальная зона ингибирования (18 мм) бактерий *Pectobacterium carotovorum* составила при концентрации 0.4 %.

**Ключевые слова:** N-фенилацетамид, дитиокарбаматы, тиоангидриды, фунгицидная и бактерицидная активность

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# N-(4-МЕТОКСИФЕНИЛ)АЦЕТАМИД ЖӘНЕ N-ФЕНИЛАЦЕТАМИД ТУЫНДЫЛАРЫНЫҢ СИНТЕЗІ, ОЛАРДЫҢ БАКТЕРИЦИДТІК ЖӘНЕ ФУНГИЦИДТІК БЕЛСЕНДІЛІГІ

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**Түйіндеме.** *Кіріспе.* Техникалық және ауылшаруашылық дақылдарын өсіру кезінде патогендік саңырауқұлақтармен күресуге ерекше назар аударылады, бұл өнімділіктің айтарлықтай жоғалуына әкеліп қана қоймай, адамдар үшін де қауіпті. Жұмыстың *мақсаты* N-(4-метоксифенил)ацетамид пен N-фенилацетамид негізінде ароматикалық дитиокарбамин қышқылдарының қатарындағы биологиялық белсенді заттарды синтездеу және олардың саңырауқұлаққа қарсы және бактерияға қарсы белсенділігін зерттеу болып табылады. *Нәтижелер және талқылау.* Биологиялық белсенді ароматикалық натрий дитиотиокарбаматтары 78 және 85% шығыммен аминдердің (N-(4-метоксифенил)ацетамида және N-фенеилацетамид) күкірткөміртекпен NaOH қатысында этил спиртінде 22 °С температурада синтезделіп алынды. Тиоангидридтер натрий ацетил(4-метоксифенил)карбамодитиоаты және натрий ацетил(фенил)карбамодитиоатының хлорангидридтермен (бензойлы, 3,4-диметоксибензойлы, 2-бромбензойлы және 4-нитробензойлы) хлороформ ортасында 22 °С температурада ацилдеу арқылы синтезделді. Синтезделген қосылыстардың құрылымы элементтік талдау, ИҚ-спектрлері, ЯМР $^{1}$Н және $^{13}$С спектроскопиясы негізінде дәлелденді. *Қорытынды.* Синтезделген қосылыстардың *Fusarium oxysporum* және *Pectobacterium carotovorum* фитопатогенді саңырауқұлағына және бактериясына саңырауқұлаққа қарсы және бактерицидтік қасиеттері зерттелінді. Бақылаумен және тетраметилтиурамдисульфид (ТМТД) және Фитолавин эталондарымен салыстырғанда натрий ацетил(4-метоксифенил)карбамодитиоаты жоғары фунгицидтік және бактерицидтік белсенділік көрсеткені анықталды. Сынақтар нәтижесінде натрий ацетил(4-метоксифенил)карбамодитиоаты 0.4% концентрациясында жоғары фунгидцидті белсенділікке ие екендігі және Fusarium oxysporum фитопатогенінің өсімін толықтай тежейтіні анықталды. *Pectobacterium carotovorum* бактериясының максималды тежелу аймағы (18 мм) 0.4 % концентрацияда болды.

**Түйін сөздер:** N-фенилацетамид, дитиокарбаматтар, тиоангидридтер, фунгицидтік және бактерицидтік белсенділік

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