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Antibacterial Activities of Coordination Compounds Containing Dicyanidoargentat (I)

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Abstract: Our study is on testing of different concentrations (5, 10, 15, 20, 30 and 40 µg/ml) of twelve the newly synthesized bimetallic cyanido complexes ([Ni(*hydeten*)2Ag(CN)2] [Ag(CN)2].H2O (K1), [Cd2(*hydeten*)2Ag4(CN)8].H2O (K2), [Ni(*bishydeten*)2Ag(CN)2] [Ag(CN)2].H2O (K3), [Cu(*bishydeten*)2Ag2(CN)4] (K4), [Cd(*bishydeten*)0.5]2[Ag(CN)2]4.3H2O (K5), [Ni(N-*bishydeten*)Ag3(CN)5] (K6), [Cu(N*bishydeten*)Ag3(CN)5] (K7), [Zn(N-*bishydeten*)Ag3(CN)5] (K8), [Cd(N-*bishydeten*)]4[Ag(CN)2]8[Ag(CN)]2 (K9), [Cu(*edbea*)Ag2(CN)4].H2O (K11), [Cd(edbea)2][Ag(CN)2]2.H2O (K12) and [Cd(edbea) Ag3(CN)5] (K13) for their antibacterial activity against *Pseudomonas syringae* pv. *tomato* (*Pst*), *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) and *Xanthomonas axonopodis* pv. *vesicatoria* (*Xav*). Antibacterial activity determined using the agar well diffusion assay method.showed that compound K9 has by 90% inhibition ratio on the bacterial growth . Eight out of 12 compounds has over 90% inhibition on *Cmm.* Inhibitory effects varying between 72% and 100% ratio by K11 and K12 on selected bacterial strains were less than the effects of K9 and K3 which showed highly significant antibacterial activity by 93.83% and 85.71% inhibitions against *Pst* respectively, whereas the lowest activity was by K4 (33%) treatment at 40 µg/ml against whole bacterial pathogens tested *in vitro* conditions. On the other hand compound K13 exhibited the highest antibacterial activity against *Xav* by 99% growth inhibition at 40 µg/ml. Most of these compounds showed promising activities against the tested bacteria. Our further studies will continue on testing of their efficiency under field conditions.

Keywords: Disiyanido-compounts, synthesized compounds, antibacterial,

Disiyanidogümüş (I) İçeren Koordinasyon Bileşiklerinin Antibakteriyel Aktiviteleri

Öz: Bu çalışmada yeni sentezlenmiş 12 disiyanidogümüş içeren bileşiklerin ([Ni(*hydeten*)$_2$Ag(CN)$_2$][Ag(CN)$_2$].H$_2$O (K1), [Cd$_2$(*hydeten*)$_2$Ag$_4$(CN)$_8$].H$_2$O (K2), [Ni(*bishydeten*)$_2$Ag(CN)$_2$][Ag(CN)$_2$].H$_2$O (K3), [Cu(*bishydeten*)$_2$Ag$_2$(CN)$_4$] (K4), [Cd(*bishydeten*)$_{0.5}$]$_2$[Ag(CN)$_2$]$_4$.3H$_2$O (K5), [Ni(N-*bishydeten*)Ag$_3$(CN)$_5$] (K6), [Cu(N-*bishydeten*)Ag$_3$(CN)$_5$] (K7), [Zn(N-*bishydeten*)Ag$_3$(CN)$_5$] (K8), [Cd(N-*bishydeten*)]$_4$[Ag(CN)$_2$]$_8$[Ag(CN)]$_2$ (K9), [Cu(*edbea*)Ag$_2$(CN)$_4$].H$_2$O (K11), [Cd(edbea) $_2$][Ag(CN)$_2$]$_2$.H$_2$O (K12), ve [Cd(edbea)Ag$_3$(CN)$_5$] (K13) farklı dozlarının (5, 10, 15, 20, 30 and 40 µg/ml) *Pseudomonas syringae* pv. *tomato* (*Pst*), *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) ve *Xanthomonas axonopodis* pv. *vesicatoria* (*Xav*) üzerindeki antibakteriyel etkileri belirlenmiştir. Test yöntemi olarak agar kuyucuk difüzyon yöntemi kullanılmıştır. En iyi sonuç K9 maddesinden elde edilmiş olup, bakterilerin koloni gelişimini %90 ve üzerinde engellemiştir. 12 maddeden 8 tanesinin *Cmm*'in gelişimini %90 ve üzerinde engelledikleri belirlenmiştir. K11 ve K12 maddeleri testlenen bakterileri %72 ile %100 arasında değişen oranlarda engellemişlerdir. *Pst* üzerinde K9 ve K3 maddeleri sırasıyla %93,83 ve %85,71 oranlarında etkili olurken, en düşük etki 40 µg/ml dozu ile K4 (%33) maddesinde belirlenmiştir. Diğer taraftan *Xav* üzerinde en yüksek etki %99 oran ile 40 µg/ml dozlu K13 maddesinde görülmüştür. Bu maddelerin çoğunluğunda antibakteriyel etkilerine yönelik ümit var sonuçlar elde edilmiştir. Ancak bu maddelerin tarla koşullarında etkinliklerini belirlemeye yönelik çalışmaların yapılması gerekmektedir.

Anahtar kelimeler: Disiyanidogümüş maddeler, sentezlenmiş bileşenler, antibakteriyel

***:**This study was produced from the first author's master thesis.

1. Introduction

Causal agent of bacterial leaf spot, *Xanthomonas axonopodis* pv. *vesicatoria* (L.R. Jones) Dye (*Xav*), bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye and Wilkie (*Pst*) and *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al. (*Cmm*), the causal agent of bacterial wilt and canker of tomato, are economically important and devastating diseases of pepper and tomato. The diseases occur worldwide where pepper and tomato are grown in warm, moist areas (Gleason et al. 1993; Pernezny et al. 1995; Jones and Pernezny, 2003). Different control methods are used for management of these bacterial diseases such as cultural, physical, biological and chemical control (Jones and Pernezny, 2003). However, chemical control is the common control practice for management of bacterial plant pathogen, chemical control is limited to copper or copper combined with maneb or mancozeb sprays that provide only marginal success thus making the diseases very difficult to control once the epidemic is underway (Conover and Gerhold, 1981; Hausbeck et al. 2000; Hovarth et al. 2012; Miller and Jones, 2014). Copper resistance can easily develop within these bacterial populations (Louws et al. 2001; Werner et al. 2002; Martin et al. 2004; Baysal et al. 2005).

In recent years, an increase in demand to develop alternative antimicrobial substances with lesser toxicity to the environment and non-target organisms. Recently, besides plant extracts newly synthesized synthetic chemicals are tested in control of plant pathogens (Korkmaz et al. 2014; Karadag et al. 2015, 2018, Karaca et al. 2020). Antifungal and antibacterial activities of the newly synthesized compounds have become one of the new chemistry research areas (Andriole, 1999; Topliss et al. 2002; Kumar et al. 2006). Among these substances, compounds containing dicyanidoargentate (I) have an important place.

In the study conducted by Karadag et al. (2015), anticancer, antifungal and antibacterial effects of the dicyanidoargentate (I) containing complexes have shown significant antibacterial activity on various gram-positive and gram-negative bacteria. Although studies carried out on the effects of dicyanidoargentate (I) containing compounds on different types of cancer cells and other human pathogens and on their effects on plant pathogenic fungi are present (Korkmaz et al. 2014, 2017; Karadag et al. 2015, 2018, Karaca et al. 2020) there are no studies on the effects of these compounds on plant pathogenic bacteria. We aimed present study to test the antibacterial activity of the diciyanidoargentate compounds against three important plant pathogenic bacteria.

2. Material and Methods

2.1. Dicyanidoargentate (I) Complexes

These Dicyanidoargentate (I) complexes, ([Ni(*hydeten*)2Ag(CN)2] [Ag(CN)2].H2O (K1), [Cd2(*hydeten*)2Ag4(CN)8].H2O (K2), [Ni(*bishydeten*)2Ag(CN)2] [Ag(CN)2].H2O (K3), [Cu(*bishydeten*)2Ag2(CN)4] (K4), [Cd(*bishydeten*)0.5]2[Ag(CN)2]4.3H2O (K5), [Ni(N-*bishydeten*)Ag3(CN)5] (K6), [Cu(N*bishydeten*)Ag3(CN)5] (K7), [Zn(N-*bishydeten*)Ag3(CN)5] (K8), [Cd(N-*bishydeten*)]4[Ag(CN)2]8[Ag(CN)]2 (K9), [Cu(*edbea*)Ag2(CN)4].H2O (K11), [Cd(edbea)2][Ag(CN)2]2.H2O (K12) and [Cd(edbea) Ag3(CN)5] (K13) were Synthesized by Dr. Ahmet Karadag, Department of Chemistry, Tokat Gaziosmanpasa University, Turkey, in the context of project 112 T 696 supported by The Scientific and Technological Research Council of Turkey (TUBITAK).

2.2. Plant Pathogenic Bacteria

Three plant pathogenic bacteria, *Xanthomonas axonopodis* pv. *vesicatoria, Pseudomonas syringae* pv. *tomato* and *Clavibacter michiganensis* subsp. *michiganensis* were obtained from a culture collection of Department of Plant Protection, Agricultural Faculty, Tokat Gaziosmanpasa University, Turkey. They were maintained on King B medium (King et al. 1954) and then stored in Nutrienth Broth and Glyserol at -20ºC. All the bacteria were subcultured by transferring from the stock cultures to King B medium in Petri dishes before use.

2.3. Bioaassay

Growth inhibition test was performed according to the modified Boyanova et al. (2005) method. Briefly, a 20 mL of King B medium was poured into a 9-cm diameter sterile Petri dish. After solidification of the medium, the wells (5 mm diameter) were produced in the agar with sterile cork borer. The compounds K1, K2, K3, K4, K5, K6, K7, K8, K9, K11, K12 and K13 were diluted with 50% dimethyl sulfoxide (DMSO) solution to the final concentrations of 5, 10, 15, 20, 30, and 40 µg/ml then 20 µl of the diluted samples were pipetted into each well. Suspension of the each bacterial pathogen was prepared in the sterile physiological water from a culture of 72 h on the King B medium. The optical density of bacterial suspension was adjusted to 0.2 at 600 nm to have 10^8 cfu/ml. A volume of 0.1 ml of the suspension was speared on the medium surface with sterile glass hokey stick. The plates were then incubated in the dark at 26 ± 2 ºC for 24, 48 and 72 h for *Pst, Xav* and *Cmm*, respectively. At the end of incubation periods, diameters of the inhibition zones were measured. Three replicates were used for each treatment. 50% DMSO was loaded in control plates as a negative control. Rifampicin (2.4 mg/mL) were used as a positive control. The rates of growth inhibition (GI%) were calculated by the following formula: GI%=dc-dt/dcx100 where dc is the mean inhibition zone of rifambicin treated sets and dt is the mean inhibition zone the treatment sets. Each test was run in triplicate.

2.4. Statistical analysis

Analysis of variance (ANOVA) was used to determine the effects of the chemicals on mycelial growth inhibition of fungi. Statistical analysis was performed with SPSS (version 9.1.3) statistical software (SPSS, 2007) the means were compared by the Tukey test. Inhibition zone data were analyzed using POLO-PC probit (Finney, 1971) to estimate lethal concentration 50 and 90 values (LC50 and 90) and the regression line slope.

3. Results and Discussion

Inhibition rates determined by our studies on the Dicyanidoargentate (I) complexes are in Table 1, Table 3 and Table 5. Antibacterial effects of the compound K5 increased with ascending concentration and effective dose has started by 20 µg/ml concentration and inhibition rates reached up to 100%. We observed the highest inhibition on *Cmm*. The compounds K1, K3, K12 and K13 caused 100% growth inhibition on *Cmm* at 30 and 40 µg/ml concentration. The compound K4 did not inhibit the bacterial growth up to 15 µg/ml concentration while it was resulted in 29% inhibition at 20 µg/ml. Inhibition rates of compounds K9 and K11 at 40 µg/ml concentration were 90 and 76% respectively. Inhibition rates of compound K2 was 55% at 5 µg/ml and reached upto 92% and above starting from 20 µg/ml concentration. The compound K6 caused 100% growth inhibition on *Cmm* at 20 µg/ml concentration. The compound K7 resulted in 96% growth inhibition on *Cmm* at 40 µg/ml. At the lowest concentration (5 µg/ml), the difference between inhibition rates of compounds K1, K4, K7 and K8 was statistically insignificant ($p \leq 0.05$). However, compound K13 exhibited significantly higher inhibitory effect than other compounds ($p \leq 0.05$). At 20 µg/ml concentration, compounds K1, K2, K5, K6 ve K12 exhibited similar rates of inhibition on *Cmm* but their inhibition rates were significantly higher than the other compounds tested ($p \leq 0.05$) (Table 1).

It gives the estimated LC50 and LC90 values for the inhibitory effect of the compounds on *Cmm* colony growth in Table 2. The LC50 values of the compounds range from 4 µg/ml to 22.3 µg/ml. Compound K13 had the lowest LC50 value of 4 µg/ml while compound K11 had the highest LC50 value of 22.3 µg/ml. Therefore K2, K4 and K13 are more toxic than other compounds. Since the confidence intervals of LC50 values of these compounds coincide with each other, we found the toxicity values of these compounds to be the same. The estimated LC90 of the compounds varied from 10.7 to 78.5 µg/ml. The lowest LC90 value was K5 (10.7 µg/ml) and we found the highest LC90 value in K11 (78.5 µg/ml). The LC50 and LC90 values of the substance K11 showed that *Cmm* had the lowest toxicity on colony development among the tested

substances (Table 2).

Table 1. Inhibition rates of different concentrations of the dicyanidoargentate(I) complexes on *Cmm*
Çizelge 1. *Farklı konsantrasyonlardaki Disiyanidogümüş (I) bileşiklerinin Cmm üzerindeki engelleme* oranları

Dicyanido argentate (I) complexes	The compounds concentrations/ Inhibition rates (± S.E)					
	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml
K1	0.00±0.00(a)*(A)**	76.48±1.24(b)(G)	90.15±2.10(c)(F)	97.14±2.01(d)(GH)	100±0.00(d)(E)	100±0.00(d)(E)
K2	55.10±0.96(a)(E)	75.30±0.96(b)(G)	77.71±0.74(b)(E)	92.94±1.16(c)(G)	96.87±1.95(d)(E)	100±0.00(e)(E)
K3	21.91±0.77(a)(C)	59.43±1.66(b)(DE)	71.28±0.34(c)(E)	85.21±1.36(d)(F)	100±0.00(e)(E)	100±0.00(e)(E)
K4	0.00±0.00(a)(A)	0.00±0.00(a)(A)	0.00±0.00(a)(A)	0.00±0.00(a)(A)	15.46±1.29(b)(A)	29.41±1.06(c)(A)
K5	60.22±0.22(a)(F)	85.05±0.70(b)(H)	96.26±1.52(c)(F)	100±0.00(d)(H)	100±0.00(d)(E)	100±0.00(d)(E)
K6	36.24±1.14(a)(D)	63.87±0.23(b)(EF)	74.68±0.73(c)(E)	100±0.00(d)(H)	100±0.00(d)(E)	100±0.00(d)(E)
K7	0.00±0.00(a)(A)	41.09±2.07(b)(C)	59.26±2.28(c)(D)	64.58±0.83(d)(D)	73.33±2.06(e)(D)	96.29±2.20(f)(DE)
K8	0.00±0.00(a)(A)	0.00±0.00(a)(A)	12.44±0.91(b)(B)	19.15±1.65(c)(B)	21.11±1.24(c)(B)	56.28±1.68(d)(B)
K9	51.23±1.23(a)(E)	55.53±0.83(b)(D)	63.78±0.80(c)(D)	71.72±0.33(d)(E)	73.83±0.48(d)(D)	90.40±2.53(e)(D)
K11	12.73±1.48(a)(B)	15.71±0.98(a)(B)	24.39±2.33(b)(C)	43.54±0.95(c)(C)	66.06±1.99(d)(C)	76.03±2.31(e)(C)
K12	52.95±1.19(a)(E)	69.02±2.04(b)(F)	75.44±2.33(c)(E)	95.74±1.52(d)(GH)	100±0.00(e)(E)	100±0.00(e)(E)
K13	65.51±1.40(a)(G)	68.96±1.31(b)(F)	71.15±0.00(c)(E)	85.09±0.00(d)(F)	100±0.00(e)(E)	100±0.00(e)(E)

*Means followed by the same letter in a column are not significantly different at 5% level of probability (Tukey's Multiple Rang Test)

Table 2. The estimated LC50 and LC90 values of dicyanidoargentate(I) complexes against *Cmm*
Çizelge 2.*Disiyanidogümüş(I) bileşiklerinin Cmm üzerine engelleyici etkileri ve rezidüyel toksisiteleri*

Dicyanido argentate (I) complexes	Slope (±SE)a	LC50 (95% of fiducial limits)	LC90 (95% of fiducial limits)	χ2b
K1	7.3±0.4	8.9 (8.1-9.7)	13.3 (12.2-14.9)	69.2
K2	2.4±0.1	5.0 (4.0-6.0)	17.0 (14.9-20.3)	37.8
K3	3.4±0.1	8.8 (8.0-9.6)	21.2 (19.1-24.1)	34.2
K4	3.3±0.2	4.4 (3.9-4.8)	10.7 (9.9-11.6)	12.8
K5	3.4±0.2	7.2 (6.1-8.3)	17.1 (14.8-20.7)	73.2
K6	3.2±0.1	14.2 (12.1-16.4)	35.6 (29.1-48.3)	116.7
K9	1.2±0.1	5.9 (3.9-7.7)	72.2 (49.4-137.0)	33.2
K11	2.3±0.1	22.3 (20.4-24.6)	78.5 (64.0-102.5)	27.9
K12	2.5±0.1	5.6 (4.1-6.8)	18.3 (15.2-23.6)	73.7
K13	1.8±0.1	4.0 (2.3-5.6)	20.2 (16.0-28.7)	71.4

[a]Slope of the concentration (± standarderror) response of *Cmm* to compounds.
[b]Pearson chi-square goodness-of-fit test on the probit model ($\alpha = 0.05$).

Inhibition rates of the dicyanidoargentate(I) complexes on *Pst* increased with an increase in concentration. Compound K9 caused 94% inhibition on *Pst* at 40 µg/ml. Because it was the only compound that showed over 90% inhibition at 40 µg/ml, it had the highest effect on *Pst*. The compound K4 inhibited colony growth by only 34% even at the highest concentration (40 µg/ml). The inhibition rates of compound K2, K3, K5, K6 and K13 were 84, 86, 84, 82, and 72% at 40 µg/ml, respectively. The highest inhibition rate of 53% was obtained with compound K8 at 40 µg / ml. The compounds K1, K7 and K12 were not inhibited at 5 µg / ml and caused inhibition of K1 (72%), K7 (83%) and K12 (80%) at 40 µg/ml. We found significant differences between the substances to block rates at different doses ($p \leq 0.05$). At the lowest concentration (5 µg/ml), inhibition rates of compounds K5, K6 and K9 differed significantly from other compounds. In addition, the difference between K1, K4, K7, K8, K11 and K12 was found to be insignificant ($p \leq 0.05$). The effect of compounds K5 and K9 at 30 µg/ ml concentration differed significantly from other compounds (Table 3).

When the inhibitory effects of dicyanidoargentate (I) complexes on *Pst* colony development are examined, LC50 values vary between 6.1 µg/ml and 27 µg/ml. The lowest LC50 value was exhibited by compound K6 (6.1 µg/ml) and the highest of that was exhibited by K11 (27.0 µg/ml). Consequently, compounds K3, K5 and K6 are more toxic than other compounds

tested. Since the confidence intervals of LC50 values of these substances coincide with each other, we found the toxicity values of these substances to be the same. LC90 values of of the tested compounds for *Pst* ranged from 42.3 µg/ml to 132.2 µg/ml. We obtained the lowest LC90 value with compound K9 (42.3 µg/ml) and it obtained the highest one with K6 (132.2 µg/ml). Since the confidence intervals of LC90 values of K7 and K9 were the same, we found the toxicity levels of these compounds to be the same (Table 4).

Table 3. İnhibition rates of different concentrations of the dicyanidoargentate(I) complexes on *Pst*
Çizelge 3. *Farklı konsantrasyonlardaki Disiyanidogümüş (I) bileşiklerinin Pst üzerindeki engelleme* oranları

Dicyanido argentate(I) complexes	The compounds concentrations/ Inhibition rates (± S.E)					
	5µg/ml	10 µg/ml	15 µg/ml	20µg/ml	30µg/ml	40 µg/ml
K1	0.00±0.00(a)*(A)**	39.96±0.17(b)(C)	53.34±2.17(c)(D)	63.14±1.29(d)(CD)	64.31±1.18(d)(D)	72.08±2.10(e)(C)
K2	40.94±1.87(a)(CD)	56.56±0.60(b)(E)	66.30±0.72(c)(FG)	67.23±2.51(c)(DE)	69.40±1.37(c)(DE)	84.02±0.85(d)(D)
K3	36.39±1.77(a)(BC)	61.37±1.08(b)(F)	69.82±0.68(c)(G)	71.05±1.26(c)(EF)	71.76±1.01(c)(EF)	85.71±1.25(d)(DE)
K4	0.00±0.00(a)(A)	0.00±0.00(a)(A)	0.00±0.00(a)(A)	15.57±0.76(b)(A)	29.68±1.47(c)(A)	33.79±0.58(d)(A)
K5	47.17±1.01(a)(E)	55.93±1.68(b)(E)	63.04±2.30(c)(F)	74.87±0.15(d)(F)	84.50±0.87(e)(H)	85.47±1.56(e)(D)
K6	45.82±2.07(a)(DE)	57.17±0.55(b)(E)	60.29±1.54(b)(EF)	70.52±1.01(c)(EF)	70.90±0.05(c)(E)	82.28±2.29(d)(D)
K7	0.00±0.00(a)(A)	26.60±0.90(b)(B)	46.25±0.79(c)(C)	59.89±0.08(d)(C)	77.07±1.40(e)(FG)	83.08±1.40(f)(D)
K8	0.00±0.00(a)(A)	0.00±0.00(a)(A)	0.00±0.00(a)(A)	40.27±0.87(b)(B)	44.97±0.44(c)(B)	53.35±3.51(d)(B)
K9	41.56±1.27(a)(CDE)	58.67±0.84(b)(EF)	60.04±0.44(b)(EF)	70.60±1.38(c)(EF)	82.27±1.10(d)(GH)	93.83±2.22(e)(E)
K11	0.00±0.00(a)(A)	0.00±0.00(a)(A)	33.06±1.79(b)(B)	45.19±2.32(c)(B)	54.33±1.92(d)(C)	60.39±0.44(e)(B)
K12	0.00±0.00(a)(A)	48.58±0.52(b)(D)	53.94±1.27(c)(DE)	58.72±1.92(d)(C)	67.83±1.39(e)(DE)	80.50±1.60(f)(CD)
K13	31.07±2.23(a)(B)	58.36±0.56(b)(EF)	61.66±1.52(c)(F)	64.84±1.02(d)(CDE)	71.88±0.26(e)(EF)	72.15±0.46(e)(C)

*Means followed by the same letter in a column are not significantly different at 5% level of probability (Tukey's Multiple Rang Test)

Table 4. The estimated LC50 and LC90 values of dicyanidoargentate(I) complexes against *Pst*
Çizelge 4. *Disiyanidogümüş (I) bileşiklerinin Pst üzerine engelleyici etkileri ve rezidüyel toksisiteleri*

Dicyanido argentate(I) complexes	Slope (±SE)a	LC50 (95% of fiducial limits)	LC90 (95% of fiducial limits)	χ2b
K1	2.3±0.1	17.2 (14.7-20.1)	60.5 (45.6-94.7)	82.5
K2	1.1±0.1	8.1 (6.6-9.4)	97.5 (71.2-152.6)	16.1
K3	1.4±0.1	7.6 (6.2-8.9)	66.4 (51.2-95.8)	20.3
K5	1.3±0.1	6.4 (5.3-7.5)	58.1 (47.0-77.0)	13.2
K6	1±0.1	6.1 (4.6-7.5)	132.2 (88.4-237.5)	5.5
K7	3.2±0.1	17.2 (16.1-18.4)	43.3 (38.8-49.5)	24.5
K9	1.8±0.1	7.9 (6.5-9.2)	42.3 (34.4-56.4)	30.7
K11	3±0.1	27.0 (23.5-32.4)	72.4 (54.1-118.3)	98.7
K12	2.4±0.1	15.7 (13.2-18.5)	53.4 (40.4-83.5)	96.5
K13	1.2±0.1	9.8 (7.9-11.5)	114.7 (78.3-205.7)	23.0

[a]Slope of the concentration (± standarderror) response of *Pst* to compounds.
[b]Pearson chi-square goodness-of-fit test on the probit model (α = 0.05).

The inhibitory effects of the dicyanidoargentate(I) complexes on *Xav* were increased with an increase in concentration. The compound K13 had the highest inhibitory effect (65.51%) even at the lowest concentration (5 µg/ml). We considered the compound K4 ineffective with 41% inhibition at the highest concentration (40 µg/ml). Inhibitory effects of compounds K2, K7 and K11 increased with an increase in concentration and showed a ratio of inhibition rates of 72% and above at 40 µg/ml concentration. The inhibition rates of compounds K1 and K3 increased starting at 10 µg/ml concentration, leading to 97% and 89% inhibition at the highest concentration, respectively. Inhibitory effects of compounds K6 and K9 increased with an increase in concentration and showed a ratio of inhibition rate of 90% at 40 µg/ml concentration. The K8 did not cause any inhibition up to 15% µg/ml concentration. However, this rate increased to 60% at 40 µg/ml. İnhibitor activities of the compounds different from each other at some concentration ($p \leq 0.05$). At the lowest concentration (5 µg/ml), K13

exhibited significantly higher inhibitory effect than other compounds ($p \leq 0.05$). The difference among inhibitory effects of K1, K3, K5, K9 and K12 was found to be statistically insignificant at 20 µg/ml. The growth inhibition rate of K13 was significantly higher than other compounds at 20 µg/ml ($p \leq 0.05$) (Table 5).

Table 5. Inhibition rates of different concentrations of the dicyanidoargentate(I) complexes on *Xav*
Çizelge 5. *Farklı konsantrasyonlardaki Disiyanidogümüş (I) bileşiklerinin Xav üzerindeki engelleme* oranları

Dicyanido argentate(I) complexes	The compounds concentrations/ Inhibition rates (± S.E)					
	5µg/ml	10µg/ml	15µg/ml	20 µg/ml	30 µg/ml	40 µg/ml
K1	0.00±0.00(a)*(A)**	52.45±1.73(b)(D)	60.71±0.89(c)(CD)	81.14±2.41(d)(D)	85.40±0.72(e)(CD)	96.74±1.15(f)(FG)
K2	29.40±1.17(a)(C)	46.49±1.41(b)(C)	60.35±2.17(c)(CD)	67.55±1.18(d)(C)	72.87±2.17(e)(B)	73.22±2.12(e)(C)
K3	0.00±0.00(a)(A)	19.19±2.08(b)(B)	63.90±1.38(c)(DE)	80.57±0.11(d)(D)	81.46±1.35(d)(C)	88.86±1.36(e)(DE)
K4	0.00±0.00(a)(A)	0.00±0.00(a)(A)	0.00±0.00(a)(A)	14.68±0.71(b)(A)	23.70±2.00(c)(A)	41.50±0.58(d)(A)
K5	45.72±0.78(a)(F)	64.72±0.55(b)(F)	74.47±0.86(c)(F)	81.00±1.14(d)(D)	85.43±0.62(e)(CD)	85.50±1.32(e)(D)
K6	35.75±1.26(a)(D)	57.66±1.29(b)(DE)	69.48±0.81(c)(EF)	77.76±0.78(d)(D)	82.71±0.98(e)(C)	90.11±0.95(f)(DEF)
K7	13.79±0.47(a)(B)	46.11±0.45(b)(C)	54.36±1.32(c)(C)	58.92±0.40(d)(B)	70.73±1.72(e)(B)	75.51±2.24(f)(C)
K8	0.00±0.00(a)(A)	0.00±0.00(a)(A)	6.51±2.34(b)(A)	12.81±1.07(c)(A)	21.74±1.25(d)(A)	56.91±1.83(e)(B)
K9	41.11±0.77(a)(E)	61.18±0.59(b)(EF)	68.99±2.07(c)(EF)	77.78±1.78(d)(D)	86.00±1.05(e)(CD)	90.33±0.31(f)(DEF)
K11	10.89±1.09(a)(B)	17.45±0.55(b)(B)	29.77±0.30(c)(B)	54.23±2.54(d)(B)	67.17±1.39(e)(B)	72.10±2.01(f)(C)
K12	0.00±0.00(a)(A)	59.36±0.98(b)(EF)	68.12±1.27(c)(EF)	79.36±1.05(d)(D)	91.01±0.88(e)(DE)	93.06±0.30(e)(EFG)
K13	61.52±0.86(a)(G)	72.37±1.62(b)(G)	83.77±1.31(c)(G)	90.63±3.10(d)(E)	92.52±0.16(d)(E)	99.01±0.62(e)(G)

*Means followed by the same letter in a column are not significantly different at 5% level of probability (Tukey's Multiple Rang Test)

When considering the inhibitory effects of dicyanidoargentate(I) complexes on colony development of *Xav*, estimated LC50 values ranged from 3.8 µg/ml to 20.9 µg/ml (Table 6). The lowest estimated LC50 values was obtained in K13 (3.8 µg/ml) and it obtained the highest one in K11 (20.9 µg/ml). Here, compound K5 and K13 seems to be more toxic than other compounds. Since the confidence intervals of LC50 values of these substances coincide with each other, we found the toxicity levels of these compounds to be the same. Moreover estimated LC90 values ranged from 20.4 µg/ml to 89.4 µg/ml. The lowest estimated LC90 values was obtained in K13 (20.4 µ /ml and it obtained the highest one in K2 (89.4 µg/ml). Since the confidence intervals of LC90 values of K1 and K12 coincide with each other, we found the toxicity levels of these compounds to be the same. Based on the estimated LC50 and LC90 values compound K13 has higher toxicity than other compounds tested (Table 6).

Table 6. The estimated LC50 and LC90 values of dicyanidoargentate(I) complexes against *Xav*
Çizelge 6. *Disiyanidogümüş (I) bileşiklerinin Xav üzerine engelleyici etkileri ve rezidüyel toksisiteleri*

Dicyanido argentate(I) complexes	Slope (±SE)a	LC50 (95% of fiducial limits)	LC90 (95% of fiducial limits)	χ2b
K1	3.7±0.2	12.1 (10.5-13.6)	27.0 (23.3-33.1)	90.2
K2	1.4±0.1	11.2 (10.0-12.4)	89.4 (70.5-122.0)	12.7
K3	3.9±0.2	15.0 (13.4-16.7)	31.8 (27.6-38.6)	85.0
K5	1.4±0.1	5.3 (4.3-6.3)	43.3 (36.3-54.5)	4.6
K6	1.8±0.1	7.6 (6.7-8.5)	40.6 (35.3-48.3)	2.1
K7	1.9±0.1	14.7 (13.3-16.1)	71.3 (58.4-92.3)	19.7
K9	1.7±0.1	6.7 (5.9 -7.6)	39.9 (34.4- 48.0)	2.6
K11	2.3±0.1	20.9 (19.0-23.2)	77.6 (62.5-103.8)	30.8
K12	3.5±0.1	11.6 (10.0-13.0)	26.6 (22.9-32.6)	87.2
K13	1.7±0.1	3.8 (2.9-4.6)	20.4 (17.9-23.9)	18.2

[a]Slope of the concentration (± standarderror) response of *Xav* to compounds.
[b]Pearson chi-square goodness-of-fit test on the probit model ($\alpha = 0.05$).

The results of present study are correspondence with the previous antibacterial studies conducted by various researchers (Jose et al. 2001; Faimali et al. 2003; Garaventa et al.

2003; Turk et al. 2008; Kirilmiş et al. 2009). In the study conducted by Zovko et al. (2012), 3-alkylpyridinium (3- APS) analogs compounds, extracted from sea sponges, were synthesized and tested against *Candida albicans, Saccharomyces cerevisiae* and *Wallemia sebi*. They reported that *S. cerevisiae* was determined as the most sensitive species to the compounds. Similarly, thiosemicarbazone compounds were tested on *Bacillus cereus, Staphylococcus epidermis*, *Moraxella cattarhalis* Staph. *Candida albicans* and *Aspergillus flavans* and these compounds exhibited antimicrobial activities against testing microorganisms (Parul et al. 2012). Korkmaz et al. (2014) tested two different diciyanido argentate comlexis, coded C1 and C2, on *Candida utilis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Streptococcus pyogenez*, *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella gallinarum*, *Pseudomonas aeruginosa*, *Salmonella enteridis.* C1 exhibited better antibacterial activities than C2 against test bacteria. In another study, hydeten complexes with dicyanidoaurate (I), coded C1, C2, C3, C4 and C5 tested agains *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella enteridis, Pseudomonas vulgaris, Klebsiella pneumonia, Staphylococcus aureus*, *Enterococcus faecalis, Bacillus subtilis, Bacillus cereus ve Candida albicans*. We reported that C4 and C5 had higher antibacterial activities than the other compounds and they have potential to be an antibacterial drugs (Karadag et al. 2015).

In conclusion, the antibacterial activities of 12 diciyanidoargentate (I) complexes against plant pathogenic bacteria have been first showed in this study. Both the results of the previous studies and the results of the present study reveal that the compounds of the diciyanidoargentate complexes have the potential to be developed as bactericides. In addition, in vivo studies will be conducted on antibacterial activities of these compounds against plant pathogenic bacteria and their behavior in the soil and plant.

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