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Preparation, Spectroscopic Investigation Of Some Tryptophan Complexes And Their Biological Activity On The Powdery Mildew Disease

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Abstract

Three of metal complexes were prepared by modified and simple reactions between Tryptophan (as ligand) with some metal ions of : Co(II), Pb(II) and Cd(II). Several physical techniques were used to investigated the complexes, including molar conductance measurements, colour and melting point studies, and infrared investigation spectra. The complexes were subjected to thermogravimetric analysis (TGA). The biological activity of three complexes was investigated on the powdery mildew disease which were caused by *Erysiphe spp fungi* were studied.

Key word: **Tryptophan, Complexes, IR, TG, powdery mildew disease, *Erysiphe spp fungi***

Introduction

Amino acids are molecules with an amine group, a carboxylic acid group, and an additional chain that differs amongst amino acids. In biochemistry, these molecules are particularly important[1].

Powdery mildew is very important disease of Cucurbit crops with different symptoms and epidemiology. Powdery mildew fungi are obligate parasites (organisms that are totally dependent on living host for its nutrients), with spores as their reproductive structures[2]. The disease is caused by fungus *Erysiphe spp* from the family *Erysiphaceae* less common on Cucumber and melon due to the prevalence of resistant cultivars[3].

The majority of transition metal – amino acid complexes are biologically active and have a wide range of applications. Some of them have been tested for anticancer activity, including Pt(II) and Pd(II) complexes of S-2-aminoethyl-L-cysteine and S-2-aminoethyl-D,L-penicillamine hydrochloride salts[4].

Amino acids complexes have considerable biological activity, such as antitumor properties and Mixed ligand complexes of Zn(II) and Cd(II) containing ceftriaxone antibiotic and amino acids were synthesized and characterized by elemental analysis, spectroscopic, biological, and thermal analyses [5-10].

Four metal complexes with a Schiff base derived from (3-methoxy-4-hydroxy-benzaldehyde with leucine) have been produced and studied using a variety of physical techniques, including molar conductance measurements, color, melting point, infrared, and electronic spectra. Antimicrobial activity of the Schiff base and its complexes was tested against gram-positive bacteria and *Rizopus* as a fungus[11].

Some of amino acids complexes were prepared. On the produced complexes, many physical techniques were used to characterize them, including molar conductance measurements, color and melting point in addition to determination of metal contents and the infrared investigation spectra were carried out on the synthesized complexes. Antimicrobial activity of the amino acid and its new complexes was investigated[12].

Transition metal amino acid complexes have gotten a lot of interest recently because they've proven to be effective antimicrobial activity agents applied against bacterial and fungus for humans and animals, etc [13].

The present paper aims to prepare Co (II), Pb (II) and Cd (II) complexes with L-Tryptophan amino acid and illustrate their geometrical structures by using different techniques, as well as to study their biological activities, such as antifungal activity on Cucumber and Melon plants (the powdery mildew disease which were caused from *Erysiphe spp* fungi).

Experimental section

Materials

All of the chemicals used in this study were reagent grade. They including : L-Tryptophan was used a ligand , the metal salt which use to complexation with L-Tryptophan including $Co(NO_3)_2.H_2O$, $Pb(NO_3)_2$, $Cd(NO_3)_2.4H_2O$, some solvents were used in this including , ethanol (C_2H_5OH) , sodium hydroxide (NaOH), ammonium solution. Double distilled water were used throughout the experiment.

Ligand

The amino acid L-tryptophan is used as a ligand, and its structure is represented in the following Figure:

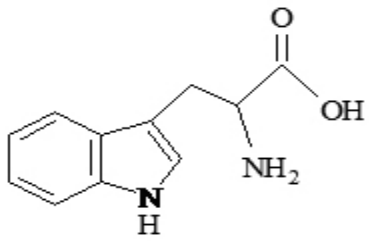


Figure (1): Structure of L-Tryptophan amino acid

Preparation of complexes

The complexes under investigation were prepared by mixing 25cm^{3}ammonium solution of the Tryptophan (0.02 moles; 4.08 g) with the same amount of ammonium solution

of the metal salts (0.02 mole); $Co(NO_3)_2.H_2O$ (5.82 g), $Pb(NO_3)_2$ (6.6 g) and $Cd(NO_3)_2.4H_2O$ (6.1 g). The obtained mixtures were refluxed with stirring for 1.5 hours, the mixture leaving in darkened place even of two day. The obtained products were filtered , and dried in desiccators over anhydrous $CaCl_2$ under vacuum. The yield ranged from 70-85% and the melting points of all complexes are above 350 ^{0}C.

Measurements

At Omar El-Mukhtar University's central lab, the conductivity values of the prepared complexes were measured using a (conduct meter) type HANA conductometer. The melting point was measured by using machines type (Melting point Apparatus SMP3). The infrared spectra of the Ligand and their metal complexes were recorded in potassium bromide discs using the IR (Type thermo FT-IR 380Nicolet company) spectrophotometer covering the range from 500 to 4000 cm^{-1}. The thermo gravimetric analysis (TGA) of some amino acids complexes which contain water molecules was achieved by using thermal technique model TGA-H50 shimadzu (Japan), the weight lost of sample was measured from room temperature up to (1000 ^{o}C) in rate of 10 ^{o}C per min, at Alexandria University, Alexandria - Egypt.

Biological applications test:

A laboratory experiment was conducted on Cucumber and Melon plants aged 14 days, at 25 ^{0}C ± 2. Powdery mildew disease caused by *Erysiphe spp* fungus was reduced using the three Co(II), Pb(II), and Cd(II) complexes. From each complex, two concentrations were selected (0.01, 0.05 mg). after treating the plants with fungi, stated earlier, the plants were widespread by the three complexes. The readings were obtained seven days later, and the results was taken according the infection stairs author's EPPO method [14] to calculate the ratio infection.

Results and Discussion

The physical properties of the L-Tryptophan and its complexes.

Table (1) gives the color and molar conductivity of the L-Tryptophan as Ligand and its complexes.

Table (1): physical properties and molar conductance of the complexes.

Complexes	Color	M .p (c^0)	E.C $sm^{2\ mol-1}$
L-Tryptophan Ligand	white	290	7.3
Co(II) complex	Dark brown	<350	0.64
Pb(ll) complex	White	<350	0.12
Cd(II) complex	Light white	<350	0.43

Table (1) showed the color of ligand was change from white color of free ligand to several different colors according to the type metal, this change mainly due to the effect

the linkage between the Ligand and for to the different of electrons in 3d orbital's[11]. The results of the melting point of the studied complexes showed different values between the free ligand and complexes, this different mainly attributed to the bounded between the metals and the ligand [15]. The conductance measurements of the prepared complexes were carried out in DMF solvent and the obtained values (Table 1) were taken as a good evidence for the existence of a non-electrolyte nature[16].

IR spectra studies:

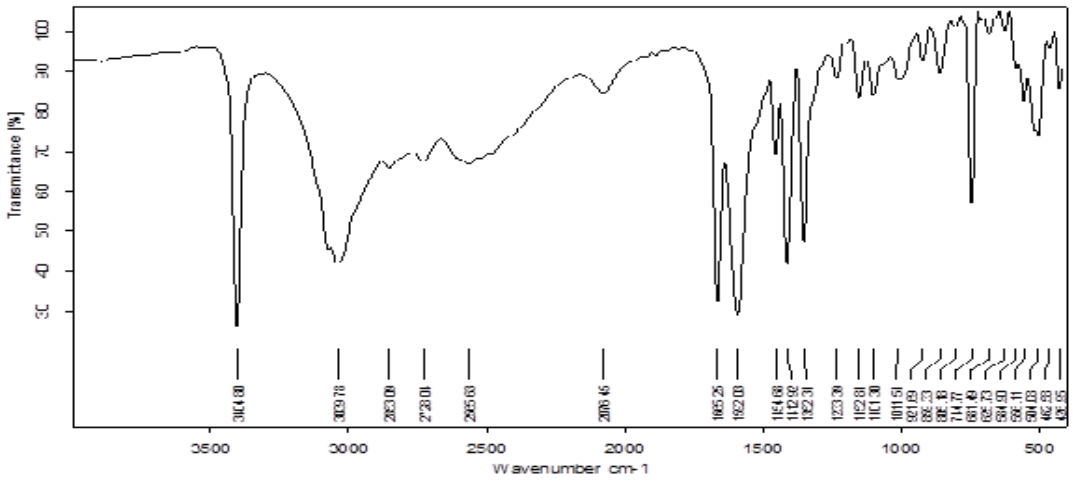
The (IR) spectroscopy approach is one of the most effective methods for examining the characterization of complexation between the ligand and the metal salts because metal salts do not give spectra, but when metals conjugated with ligands, the complex gives IR spectra [11]. Infrared spectra were used to define the structure of the produced complexes, with the metal complexes' IR spectra being assigned by comparing their vibration frequencies to those of the ligand: The infrared spectrum of Tryptophan and its complexes was provided for comparison and to aid in the complexes' spectra assignment. The obtained data are presented in Table (2) below.

Table (2): The fundamental bands of the free Tryptophan and its complexes

Complexes	C-H Aromatic	C-H Aliphatic	C=N	C=O	C=C	CH_3	C-O	C-N	N-H	M-O	M-N
L-Tryptpphan Ligand	3039	2853	1592	1665	1592	1352	1233	1152	3404	-	-
Co(II) complex	3071	2924	1623	-	-	1383	1231	1096	3402	665	426
Pb(II) complex	3294	3049	1662	-	1513	1386	1243	1084	3398	680	424
Cd(II) complex	3252	3038	1663	-	1587	1387	1224	1095	3322	587	423

The main peaks of the IR spectra are shown in Figures (2-5), as well as the most important absorption bands. In the ligand's spectrum (Tryptophan), the band of Ligand (Tryptophan) 3039 $cm^{-1}$are assigned to C-H aromatic, the first band of the Tryptophan shifted to higher frequency in Co, Pb and Cd complexes. Whereas, the same spectra display a band at 2853 $cm^{-1}$of Tryptophan are assigned to C-H aliphatic shifted to higher frequency in all complexes. Also the absorption band at at 1592 cm^{-1} of Tryptophan assigned to C=N, the band are shifted to higher frequency in Co(II), Pb(III) and Cd (II) complexes compared to its position in the original ligand (1592 cm-1) indicating its involvement in coordination with the metal ions [17]. Whereas, the absorption band at (1665 cm^{-1}) was attributed to the carbonyl stretching vibration in the ligand were disappeared in the spectra of all complexes suggests its participation as imines group in complexation [18]. The band C=C of Ligand appear in 1592 cm^{-1} shift to lower frequency in Pb(II) and Cd(III) complexes. Also the absorption band CH_3 appear at 1352 cm^{-1} shifted to higher frequency in all complexes. Whereas, the absorption band at 1233 cm^{-1} due to υ C-O vibration of the ligand is shifted to lower frequency except in case Pb(II) complex are shifted to higher frequency indicating its involvement in coordination with the metal ions through the oxygen atoms [19,20]. The υ N-H

band located at 3404 cm^{-1} are shifted to lower frequency in all complexes. New bands observed at 587-680 cm^{-1} and 423- 426 cm^{-1} which are not seen in the spectrum of the free ligand can be attributed to υ (M-O) and υ (M-N) vibrations, respectively [21].



Figure(2): I.R. Spectra for Tryptophan

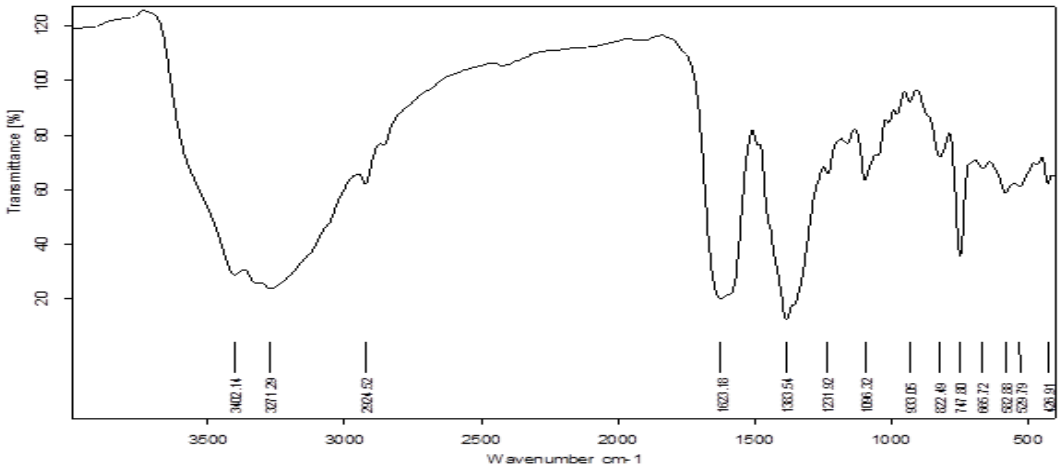


Figure (3): I.R Spectra of Tryptophan with Co(II)

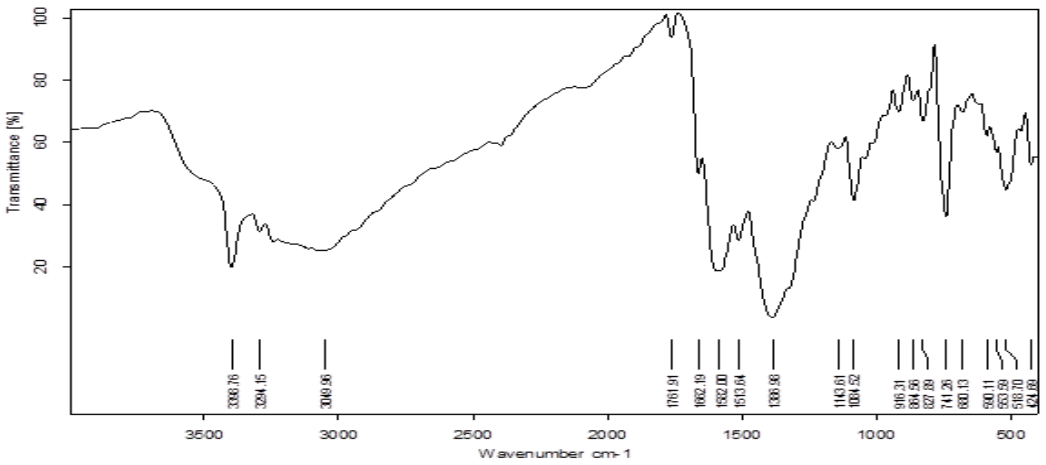


Figure (4): I.R Spectra of Tryptophan with Pb(II)

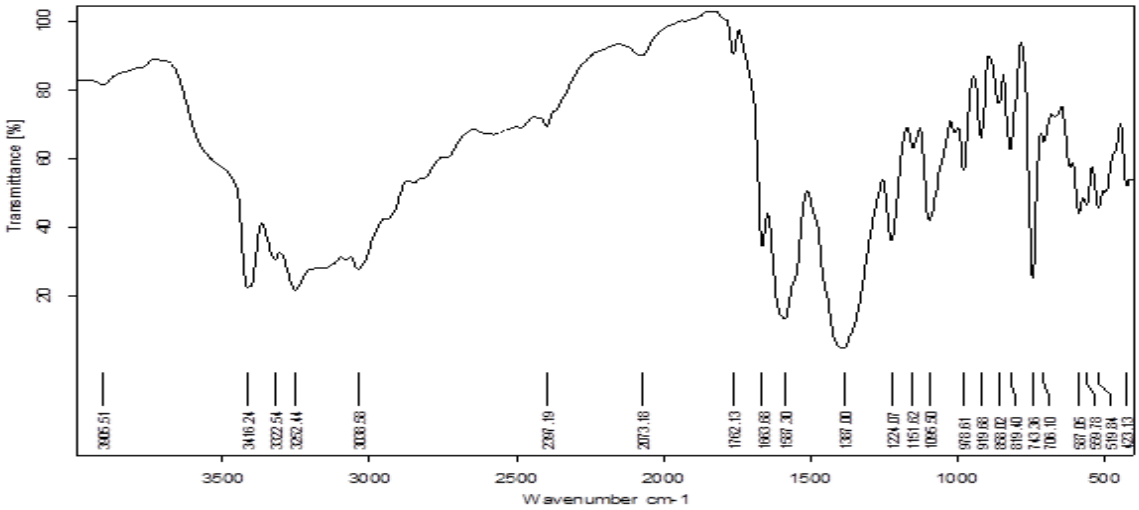


Figure (5): I.R Spectra of Tryptophan with Cd(II)

Thermogravimetric Analysis (TGA):

The weight losses were calculated using a heating rate of 10 ^{o}C/min from ambient temperature up to 1000 ^{o}C [22], and the thermogravimetric analysis of the complexes was used to aid in the prediction of molecular structures. Using thermogravimetric analysis curves, we were able to determine the temperature at which the characterized compound has a constant weight and begins to decompose, as well as how far the decomposition reaction can progress [23]. The observed and stoichiometric weight decreases of the material allow for the determination of an intermediate product produced during decomposition, as well as the temperature range within which this intermediate has constant weight[24].The thermogravimetric analysis data for some tryptophan complexes are given in Table (3). Some examples of TGA curves of the prepared complexes were presented in Figures (6 - 9). The weight loss of Tr- Co, Tr- Pb and Tr- Cd complexes corresponding to loss of CO_2 molecules at the temperature range of (307.8 to 333.4 $^{\circ}$C)[25]. While the residual of metals oxides (MO_2) of (663.4– 699.9°C) rang was appeared.

Table (3): The thermogravimetric analysis data for tryptophan complexes.

Complexes	**Decomposition**				
	Mass	CO_2 Temp. ^{0}C	Mass change	MO_2 Temp. ^{0}C	Mass change
Co(II) complex	18.75 mg	311.2	- 4.32 mg	699.9	- 9.49 mg
Pb(III) complex	15.00 mg	333.4	- 5.32 mg	663.4	- 3.60 mg
Cd(II) complex	13.09 mg	307.8	- 4.87 mg	691.3	- 3.39 mg

Based on the information presented above. The following thermal decomposition scheme may be proposed for the complexes under investigation:

$[ML.nH_2O].nH2O \rightarrow [ML.nH_2O]$

$[ML.nH_2O] \rightarrow [ML]$

$[ML] \rightarrow$ intermediate (unstable)

intermediate $\rightarrow$ Metal oxide MO_2

Intermediate (decomposition of organic ligand).

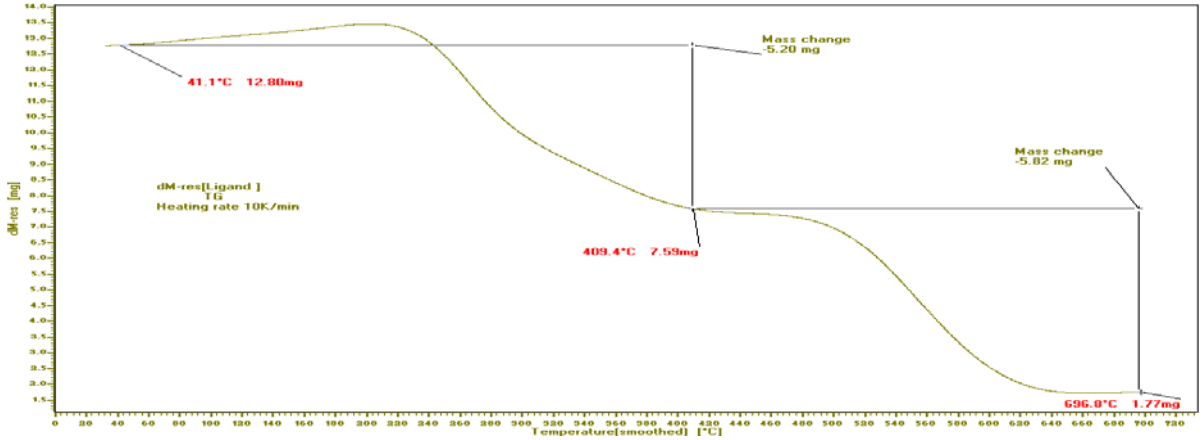


Figure (6): The TGA curve of Ligand

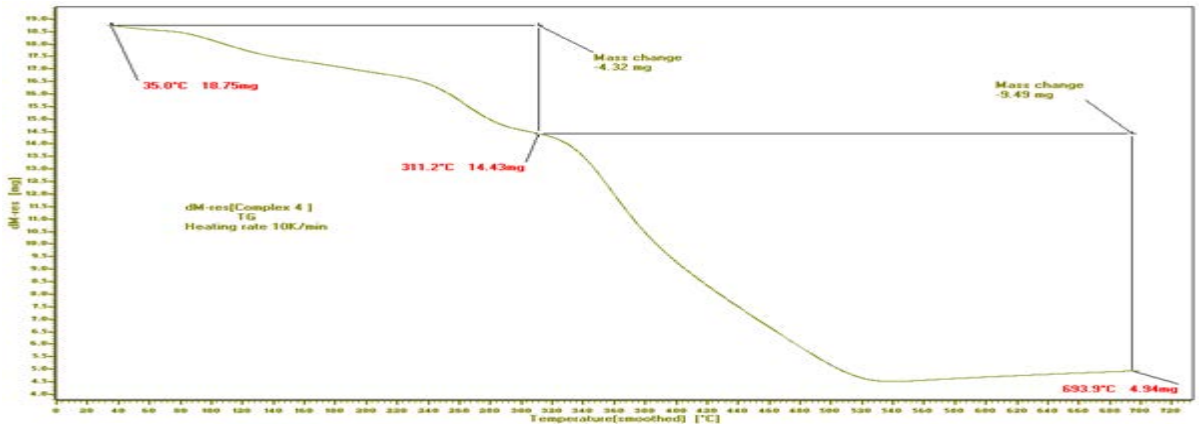


Figure (7): The TGA curve of Co(II) complex

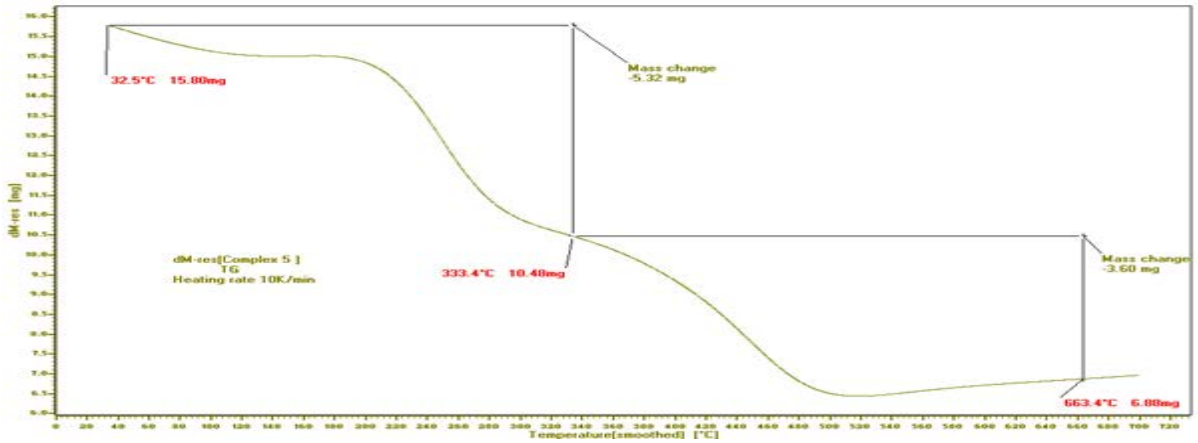


Figure (8): The TGA curve of Pb(II) complex

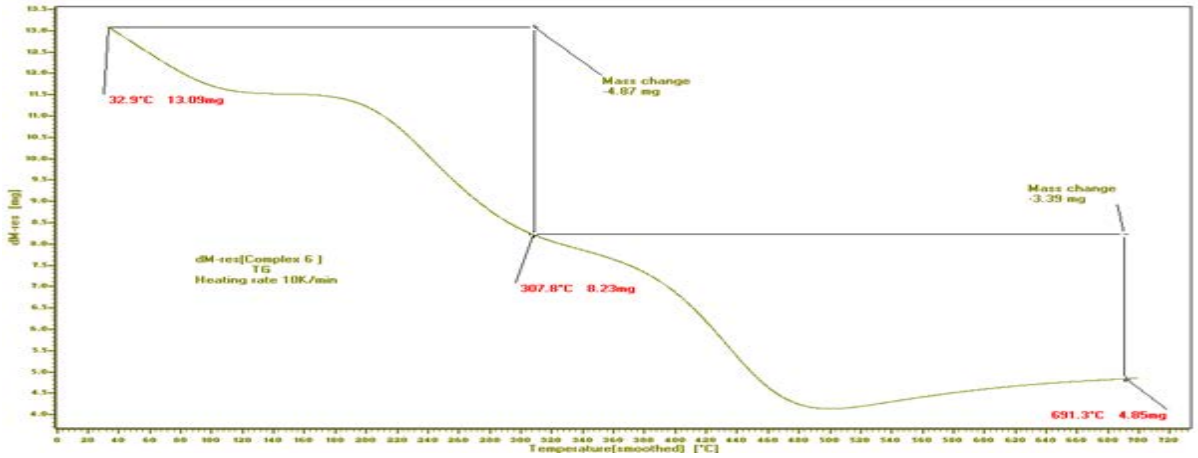


Figure (9): The TGA curve of Cd(II) complex

Biological activity test:

Effect of the three complexes on cucumber and melon plants showed in Table (4) and Figures (10-12). The result showed that, the complexes in all its concentrations have reduce the infection in comparison with the control (plant was treated only the fungi *Erysiphe spp fungi*). The most important thing is that there was no effect of the complexes on the leafs treated by complexes, while the effect was on the fungus only, so we recommend the use of these complexes as fungicide.

Table (4): The biological activity of the prepared complexes.

Treatment	complexes						
	Co(Il) complex		Pb(Il) complex		Cd(ll) complex		control
	0.01 mg	0.05 mg	0.01 mg	0.05 mg	0.01 mg	0.05 mg	
Cucumber infected by Erysiphe spp%	0	0	0	0	0	0	65.2
Melon infected by Erysiphe spp %	0	0	8.8	0	0	0	51.6

Figure (10): Control

Figure (11): Effect of Co(II), Pb(II) and Cd(II) complexes on Cucumber

Figure (12): Effect of Co(II), Pb(II) and Cd(II) complexes on Melon

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