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Effect of plant growth regulator, gibberellic acid(GA_3) and antibiotic, pencillin in the multiplication of tomato mosaic virus (ToMV)

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ABSTRACT

This experiment was conducted in Biology Department, Science College, University of Al-Anbar in 2018 season, to test the inhibition activity of gibberillic acid and antibiotic, pencillin against tomato mosaic virus.Two compounds were used to investigate resistance induction in tomato plants against the tomato mosaic virus (ToMV). In this study 50 ppm and 100 ppm of gibberillic acid and pencillin respectively were used to identify the activity in the inhibition of virus replicating or inducing the resistance in tomato plants against this virus. The results showed that the concentration 100 ppm of gibberillic acid is the best in the inhibition ratio (in the experiment of mixture tomato mosaic virus inoculum and gibberillic acid or pencillin). It gave inhibition ratio 67.44 % (lesions number, 0.8133 lesion.cm^{-2}), while the concentration 50 ppm gave inhibition ratio 54.92 % (lesions number 1.126 lesion.cm^{-2}), Moreover, the concentration 100 ppm of pencillin gave inhibition ratio 79 % (lesions number, 0.580 lesion.cm^{-2}) in comparison with the concentration 50 ppm which gave 74 % (lesions number 0.712 lesion.cm^{-2}).

The results also showed, when treating tomato plants with the concentration 100 ppm of gibberillic acid by spray on plant, gave inhibition ratio 56.44 % compared with the concentration 50 ppm which give inhibition ratio 33.85 %. Also, the pencillin treatment gave better activity in the concentration 100 ppm which give inhibition ratio 70.494 % , while the concentration 50 ppm gave inhibition ratio 20.038 %.

Based on the results of the experiment of immersion tomato roots with gibberellic acid at the concentration 100 ppm was the best, giving inhibition ratio 52.03 % while the 50 ppm of gibberellic acid gave inhibition ratio 26.89 %. Furthermore, when treating the tomato plant with pencillin the resistance against virus was higher at the concentration 100 ppm than at the concentration 50 ppm, which resulted in the inhibition ratios 63.60 % , 41.59 % respectively.

The experiment of immersion Datura roots with gibberellic acid and pencillin showed no differences between the concentration 50 ppm of gibberellic acid and control. However, the 100 ppm of gibberellic acid gave inhibition ratio 53.76 %. This experiment did not identify any differences between the antibiotic pencillin concentrations, where the concentration 100 ppm of pencillin gave the highest resistance in plant against the virus with the lowest local lesions on Datura plant (inhibition ratio, 66.267%) compared with the concentration 50 ppm which gave inhibition ratio, 21.881% and control.

Introduction:

Tomato plant *Lycopersicon esculentum* is one of the flowering and dicots plants, it is classified under solanaceae, it has an economic and food importance (1). It contains important vitamins, pigments and metal elements which are considered a major source of antioxidants (2) and are necessary to build the human body. But this important crop is getting infected with many viral diseases, including tomato mosaic virus which is affecting this crop in many countries (3). Thus, researchers resorted to use chemotherapy, which is one of the important methods utilized to resist the tomato mosaic virus. Use of plant growth regulators that lead to reduce the impact of viruses on plants (4), for example Gibberellic acid has an influential impact on the effectiveness of tomato mosaic virus on tobacco and tomato plant (5). Using antibiotic such as antibiotic chloramphenicol and pencillin has an important role in the inhibition of cucumber mosaic virus (6).

Due to the importance of the tomato crop and the gravity of the damage caused by the virus to agricultural crops, this search aims to reduce the damage caused by the virus through:

1- Inhibition of the multiplication of the tomato mosaic virus using the growth regulators, gibberellic acid.
2- Inhibition of the multiplication of the tomato mosaic virus by using antibiotic, pencillin.
3- using, plant growth regulator (GA3) and antibiotics (pencillin) to stimulate resistance in tomato plant against tomato mosaic virus .

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Material and Methods

Sterilization of peatmoss and soil:

Using, autoclave at 121ºc and press 1.5 bar to sterilize the soil and peat moss used for agriculture for at least 45 minutes and leave it for one week before use in agriculture .

Sterilization of pots:

The pots which have been used in agriculture were sterilized by using hypochlorite at a concentration of 5 % for 20 minutes and then washed with plenty of running water to remove traces of detergents .

Soil preparation and plant cultivation:

The seeds of datura and tomato, which were previously sterilized and washed and planted in pots with an upper diameter of 12 cm and the height of 11 cm, was filled with a mixture of soil and peat moss at 1:2 and then grown in a plastic house with a height of 1.5 m, length 3 m, heat 28 – 30 ºc and the plants were watered as needed, and fertilizer N.P.K. was added at concentration of 3 g/ l of water and 3 times every two weeks (7). An insecticide erotox sniper 10% was used to fight insects at least every week .

Plants which used in the present research:

The seeds of the tomato *Lycopersicon esculentum* were obtained from the local markets in Ramadi (Iraq) in packed boxes. The seeds of *Datura stramonium* were obtained from the general company for seed certification, college of agriculture, University of Baghdad .

Isolation the tomato mosaic virus:

Tomato mosaic virus was isolated from infected tomato plants that have the symptoms of the infection of mosaics. The juice of these plants was

taken and datura plants were inoculated to ensure the presence of the virus and the emergence of local lesion on the leaves, which is a characteristic of the virus. After that take one spot to be used in the inoculation of healthy tomato plants. The emergence of the symptoms of the virus on these plants is used as the first source of the vaccine tomato mosaic virus to fertilize the experimental plants later **.**

Method of preparing the tomato mosaic virus vaccine :

Smear 1 g of tomato leaves, which are infected by tomato mosaic virus and have the symptoms of infection in a ceramic mortar with 5 ml distilled water(8). The resulting juice was then filtered through 4 layers of medical gauze and the filtration of the filter was carried out with Centrifuge. The juice was then kept in glass bottles with the size of 2 ml, and then kept in the freezer to be a source of the virus vaccine used in subsequent experiments **.**

Mechanical inoculation process:

After the preparation of the virus vaccine from the infected plant, carborundum 400 mesh was sprayed on the leaves to be vaccinated with a light layer by hand dispenser prepared for this purpose, and then dip the index finger from the right hand in the vaccine and wipe the leaf with light pressure and preferably the leaf from lower by the palm of the left hand and after the completion of the inoculation, wash the surface of the leaf with water directly for a short period that does not exceed twenty seconds (9) **.**

Calculation of areas of inoculated leaves: -

The complete and incomplete squares occupied by the image of the leaf on the graph sheet were counted according to the following equation:

leaf area (square centimeters) = number of complete squares + number of incomplete squares / 2 (10).

preparing gibberellic acid solution and antibiotic pencillin.

Prepare gibberellic acid solution and penicillin solution by adding 50 mg of gibbrellic acid or penicillin per liter of solvent (solvent of 5% methanol in distilled water) to get 50 ppm concentration and add 100 mg per liter of solvent to get concentration 100 ppm.

Methods of using gibberellic acid and pencillin:

1- Effect of mixing the viral vaccine with gebbirellic acid or penicillin in the multiplication of the tomato mosaic virus (number of lesions / cm 2) .

This experiment was conducted to study the effect of mixing the gebbirellic acid or pencillin with the viral vaccine in the incidence of infection in the datura plants. The sample of the treatment in this experiment included the mixing of gibberellic acid or penicillin at a concentration of 50 and 100 ppm and pre-prepared with the viral vaccine by 1:1. The control sample consisted of mixing the viral vaccine with distilled water and 1:1, leaving the mixture for 1 hour, and then the inoculation of the whole datura leaves is done, with three leaves for each concentration or treatment. The results were taken after 8 days from inoculation. The treatments utilized are described as follows:-

1 ml viral vaccine + 1 ml of gibberillic acid at a concentration of 50%
1 ml viral vaccine + 1 ml gibberillic acid at a concentration of 100%
1 ml of the vaccine + 1 ml of penicillin at a concentration of 50%
1 ml of viral vaccine + 1 ml of penicillin at 100%
1 mL of viral vaccine + 1 ml of distilled water (control)

2- Effect of spraying healthy tomato plants with gibberillic acid or penicillin in the multiplication of the tomato mosaic virus (number of lesions / cm 2) .

Selected homogenous plants with the age of fourth leaf. They were sprayed with a solution of gibberillic acid or penicillin with the concentrations mentioned above, After spraying, the plants were left for 24 hours and then vaccinated with the virus vaccine. After the vaccination, they were left for a week until the symptoms of mosaics appeared. After that, the viral vaccine came from these plants and was treated with datura leaves at the rate of three leaves (replicates) for each treatment and concentration. The datura plants were left for 8 days, until the symptoms of local lessions appeared, and then the area of the leaves was calculated to extract the number of lessions in centimeters per square meter of the leaf. Treatments were as follows:

1 - spraying the tomato plants with gibrelic acid at a concentration of 50%.

2- spraying tomato plants with 100%.

3 - spray tomato plants with penicillin at a concentration of 50%.

4 - spraying tomato plants with penicillin at a concentration of 100%.

5 - spraying tomato plant with distilled water (control).

3- Effect of immersion of the roots of tomato plants in the gibberillic acid or penicillin in the multiplication of the tomato mosaic virus (number of lessions / cm 2).

Five tomato plants (healthy) were extracted from the soil (with age, fourth leaf). Their roots were well washed with running water and later immersed in the solution of the gibberillic acid or penicillin with the concentrations stated above (for the control treatment, the plant was immersed in the distilled water) and left immersed for 24 hours, then transferred to distilled water. Later, one leaf per plant was vaccinated with virus vaccine and left for 7 days. After that, a viral vaccine was used to vaccinate Datura plants with 3 replicates for each treatment. The datura leaves were left for 8 days, then the leaves area was calculated to extract the number of lesions in the leaf per square centimeter. Treatments were as follows**:**

1- Immersing the roots of the plant in the gibberellic acid 50%.

2 - Immersing the roots of the plant in the gibberellic acid 100%.

3 - Immersing the roots of the plant in the penicillin concentration of 50%.

4. Immersing the roots of the tomato plant in penicillin at a concentration of 100%.

5 - Immersing the root of the tomato plant with distilled water .

4- Effect of immersion of datura roots in gibberillic acid or penicillin in stimulating the resistance in these plants.

The roots of the fifth-leaf homogenous datura seedlings (after washing them well with running water) were immersed in the gibberillic acid solution or in the penicillin antibiotic with 50 ppm and 100 ppm concentrations respectively. They were left immersed for 24 hours. The control treatment consisted of immersing datura roots with distilled water, and then transferring them to the distilled water to be vaccinated by the virus by three replicates for each treatment and concentration. They were left after the vaccination for 8 days until the symptoms appeared on the infected leaves. Then the area of the leaves was calculated to extracte the number of lesions

per square centimeter of the leaf. The treatment are stated as follows :

1 - Immerse the roots of Datura plants in the Gibberillic acid concentration of 50 ppm.

2 - Immerse the roots of Datura plants in the gibberillic acid concentration of 100 ppm.

3 - Immerse the roots of Datura plants in penicillin at a concentration of 50ppm.

4 - immerse the roots of Datura plants in penicillin concentration of 100ppm.

5 - Immerse the roots of Datura plants with distilled water (control treatment) .

Statistical analysis:

We used three replicates for each treatment and concentration, the results were analyzed according to the statistical program, SPSS by using complete randomized design CRD with significant level 0.05 ($P \leq 0.05$) **.**

The results:

1- Effect of mixing the viral vaccine with gibberillic acid or penicillin in the multiplication of the tomato mosaic virus (number of lesions / cm 2)

The results presented in Table (1) showed the number of lesions per square centimeter of the area of the leaf produced by the treatment of datura leaves with gibberillic acid or antibiotic penicillin with the tomato mosaic virus compared with the treatment of the mixing of the virus with the distilled water as a control. The concentration of 100 ppm of gibberillic acid gave 0.8133 lesions / cm 2 with a significant difference from the 50 ppm concentration, which gave the number of lesions reached to 1.126 lesions / cm2, with a significant difference with the control treatment, which gave a number of lessions reached to 2.498 lesions/cm2. The same table also shows, the concentration of 100 ppm of antibiotic penicillin inhibition of the virus and giving the number of lesions 0.580 lesion/cm2 with a significant difference with the treatment of 50 ppm of penicillin and significant difference with control treatment, which gave the number of lesions 2.763 lesion/cm^2 **.**

2 - Effect of spraying healthy tomato plants with gibberillic acid or penicillin in the multiplication of the tomato mosaic virus (number of lesions / cm^2).

The results of the statistical analysis presented in Table (2) showed the superiority of the treatment of gibberillic acid at a concentration of 100 ppm on the concentration of 50 ppm in the inhibition of the virus multiplication by giving a total of 1.163 lesion / cm2 compared with a concentration of 50ppm, which gave 1.766 lesion/ cm^2. Moreover, both concentrations were significantly higher than the control treatment, which gave a total of 2.670 lesion/ cm2. In the treatment of antibiotic penicillin, the results showed that the 100 ppm of penicillin inhibition the tomato mosaic virus and give, 0.776 lesion / cm2, followed by 50 ppm, which gave a total of 2.103 lesion / cm2 compared with a concentration of 100 ppm and with the Control which gave the number of lesion 2.630 lesion/ cm^2 .

Table (1) Number of lesions resulting from the mixing of the viral vaccine with the gibberillic acid or penicillin in the tomato mosaic virus multiplication (the number of lesions/cm 2).

Number of lesion/ cm^2		
Pencillin	Gibberellic acid	treatment / ppm
A 0.712±0.049	A 1.126 ±0.02	50
A 0.580 +_0.07	A 0.8133 ±0.08	100
B 2.763_+ 0.09	B 2.498 ±0.32	Distilled water
1.351	1.4794	The average

The numbers of similar letters are not significantly different from each other according to the

test of the least significant difference L.S.D and the level of significance 0.05

3 - Effect of immersing the roots of tomato plants in the gibberillic acid or penicillin in the stimulation of resistance against the tomato mosaic virus (number of lession / cm^2).

The data which listed in Table (3) indicated that the immersion of the roots of the tomato plants with the concentration 100 ppm of gibberillic acid stimulated the resistance against the virus by decreasing the number of lesions produced in the square centimeter of the leaf by giving a number of lesion 1.063 lesion / cm^2, they also indicated a significant difference from 50 ppm, which gave the number of lesion 1.620 lesion/cm^2, and indicated the superiority of both concentrations on the treatment of control, which gave the number of lesions reached to 2.216 lesion/cm^2 The results in the same table indicated that the concentration 100 ppm of antibiotic penicillin gave the highest resistance against the virus by a decrease in the number of lesion which gave 0.860 lesion/cm2, with significant different from the concentration 50 ppm, that gave 1.380 lesion/cm^2 and comparison with the control treatment which gave 2.363 lesion/cm^2.

4-Effect of immersion of the roots of datura plants in the gibberillic acid or penicillin in stimulating the resistance in these plants .

The statistical analysis of the results listed in Table (4) indicated that the immersion the roots of the datura plants in the gibberillic acid at 100ppm concentration reduced the number of lesion per square centimeter of the area of the leaf. This concentration gave lesions 1.043 lesion/cm^2 compared with the control, which gave a total number 2.256 lesion/cm^2, while the concentration of 50ppm of gibberillic acid gave a total 2,140 lesion/cm^2, which is a significantly different from the control treatment. The results also indicated the superiority of the penicillin at 100 ppm, which led to adecrease in the number of lessions to be 0.760 lesion/cm^2.

Table (2) Number of lesions per square centimeter resulting from the spraying of tomato plants with gibberillic acid and penicillin.

Number of lesions/cm^2		
penicillin	Gibberillic acid	The treatments / ppm
A 2.103 ± 0.10	A 1.766 ± 0.11	50
B 0.776 ± 0.12	B 1.163 ± 0.02	100
C 2.630 ± 0.15	C 2.670 ± 0.14	distel water
1.836	1.866	The average

The numbers of similar letters are not significantly different from each other according to the test of the least significant difference L.S.D and the level of significance 0.05.

Table (3) Effect of immersing the roots of tomato plants in the gibberillic acid and penicillin in stimulating the resistance against the tomato mosaic virus according to the number of lesions per square centimeter.

Number of lesions/ cm^2		
Penicillin	Gibberillic acid	treatment / ppm
A 1.380 ± 0.07	A 1.620 ± 0.01	50
B 0.860 ± 0.052	B 1.063 ± 0.02	100
C 2.363 ± 0.050	C 2.216 ± 0.066	Distel water
1.534	1.633	The average

The numbers of similar letters are not significantly different from each other according to the test of the least significant difference L.S.D and the level of significance 0.05.

On the other hand, the 50 ppm treatment gave 1.760 lesion/cm^2, and both concentrations were the best compared with the control treatment, which gave a number of lesions that reached 2.253 lesion/cm^2.

Table (4) Numbers of lesion / cm 2 resulting from the effect of immersing of the roots of the datura plants in the gibberillic acid or penicillin in stimulating resistance in these plants

Lesions/ cm^2		
penicillin	Gebberillic acid	treatment / ppm
a 1.760 ± 0.08	a 2.140 ± 0.06	50
b 0.760 ± 0.03	b 1.043 ± 0.06	100
c 2.253 ± 0.10	a 2.256 ± 0.05	Distell water
1.5911	1.813	The average

The numbers of similar letters are not significantly different from each other according to the test of the least significant difference L.S.D and the level of significance 0.05.

Discussion :

This study included, the test of gibberillic acid and penicillin in reducing the multiplication of tomato mosaic virus by using the test of its effectiveness in the inhibition of virus multiplication when mixed with the virus and treatment of plants, whether tomato or Datura plants or by stimulating resistance in tomato plants treated with gibberillic acid or antibiotic (11). Spraying growth regulators such as gibberillic acid activates growth and prevents the progression of viral disease. This is consistent with the results shown in tables 2,3,4,5 of the treatment of gibberillic acid. These results are also consistent with (12), who explained that the compounds of gibberellic acid remove the factors that cause the decline in growth in most plants such as genetic dwarfism and infection with viruses and other pathogens as the effect of gibberellins on plants to promote growth and resistance against pathogens. Al-Jumaili and Al-Fahad (5) reported that growth organizations had an effect in inhibiting the tomato mosaic virus, they mentioned the superiority of gibberellic acid on growth regulators, which gave a 57% and 42% inhibition rate for both tobacco and tomato plants, respectively, when they were vaccinated 24 hours after being treated with gibberellic acid this is consistent with the results of our study. The researcher in (13) explained that the gibberillic acid stimulated plant cells to elongation against the dwarf induced by the virus as well as increase the leaves area,consequently increasing the division of cells faster than the multiplication of the virus and thus be able to escape from the disease. This catalytic effect of resistance to infected plants and treatment with regulators have affected several mechanisms in the TMV virus, which is to reduce the pathogenicity of the pathogen, facilitate the escape of plants from the disease, increasing plant tolerance of the disease, and strength the physiological resistance of the plant. These mechanisms will result in reduction in the pathogenicity ability of the virus.

The results showed that the antibiotic has an effect on the inhibition of tomato mosaic virus as it reduced the number of lesions per square centimeter of the area of the leaves compared with the control treatment. This result is consistent with the findings of (6), which explained that the antibiotic chloramphenicol has the highest inhibitory efficiency of the virus mosaic option outside the living tissue, and then followed by antibiotic penicillin which is thought to the reason behind some of the inhibitory materials used to contain the substance of phenolic suppression of the virus. Based on the same researcher, the antibiotic has an effect on the determination of the protein N in the host ribosomes, which has an effect on the process of protein aggregation with virus nuclic acid.

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تأثير منظم النمو حامض الجبريليك GA3 والمضاد الحيوي البنسلين Penicillin في تضاعف فايروس موزائيك الطماطة Tomato Mosaic Virus

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الخلاصة

اجريت هذه الدراسة في قسم علوم الحياة، كلية العلوم، جامعة الانبار لمعرفة الفعالية التثبيطية لمنظم النمو حامض الجبريليك والمضاد الحيوي البنسلين ضد فايروس موزائيك الطماطة Tomato Mosaic Virus وكذلك دراسة امكانية استخدام هذين المركبين في تحفيز المقاومة في نباتات الطماطة ضد الاصابة بهذا الفايروس، اذ استخدم التركيزين 50 ppm و 100ppm ولكل من منظم النمو حامض الجبريليك والمضاد الحيوي البنسلين لمعرفة دورهما في تثبيط تضاعف الفايروس او تحفيز المقاومة في النباتات ضد الاصابة بالفايروس، بينت النتائج بان التركيز 100 ppm من حامض الجبريليك كان الاكفأ في نسبة التثبيط (في تجربة خلط اللقاح الفايروسي مع حامض الجبريليك او البنسلين) اذ اعطى نسبة تثبيط بلغت 67.44 % (عدد نخرات 0.8133 نخرة / سم^2) مقارنة مع التركيز 50 ppm الذي اعطى نسبة تثبيط بلغت 54.92 % (عدد نخرات 1.126 نخرة/ سم^2). وكذلك الحال لمعاملة التركيز 100 ppm من البنسلين الذي اعطى نسبة تثبيط بلغت 79 % (عدد نخرات 0.580 نخرة/ سم^2) مقارنة بالتركيز 50 ppm الذي اعطى نسبة تثبيط بلغت 74 % (عدد نخرات 0.712 نخرة / سم^2).

واظهرت النتائج بان نسبة التثبيط الناتجة من معاملة نباتات الطماطة بحامض الجبريليك رشاً على النباتات كانت الاعلى وبتركيز 100 ppm اذ اعطت نسبة تثبيط بلغت 56.44 % مقارنة مع معاملة التركيز 50 ppm التي اعطت نسبة تثبيط بلغت 33.85 % وبفارق معنوي بين المعاملات، كذلك الحال بالنسبة لمعاملة المضاد الحيوي البنسلين الذي اعطى اعلى فاعلية في التركيز 100، الذي اعطى نسبة تثبيط بلغت 70.494 % ثم تلاه التركيز 50 ppm الذي اعطى نسبة تثبيط بلغت 20.038 %.

وكانت نسبة التثبيط في تجربة غمر جذور نباتات الطماطة بحامض الجبريليك الاكفأ فيما يخص معاملة التركيز 100ppm اذ اعطت نسبة تثبيط بلغت 52.03 % تلتها معاملة 50 ppm من حامض الجبريليك التي اعطت نسبة تثبيط بلغت 26.89 % والتي كانت بفارق معنوي مع معاملة السيطرة، وكذلك الحال لمعاملة غمر جذور نباتات الطماطة بالبنسلين التي اعطت لنباتات الطماطة اعلى مقاومة ضد الفايروس وبتركيز 100 تلتها معاملة التركيز 50 ppm وبنسبة تثبيط بلغت 63.60 % و 41.59 % على التوالي.

اما في تجربة غمر جذور نباتات الداتورة بحامض الجبريليك والبنسلين فيتضح عدم وجود فرق معنوي بين معاملة التركيز 50 ppm من حامض الجبريليك ومعاملة السيطرة في حين كان للتركيز 100 ppm من حامض الجبريليك نسبة تثبيط بلغت 53.76 % ، ويتضح من التجربة نفسها بان هناك فروقات معنوية بين تراكيز المضاد الحيوي البنسلين اذ اعطى التركيز 100 ppm من البنسلين اعلى نسبة مقاومة في النباتات ضد الفايروس متمثلة بقلة عدد النخرات الناتجة على نباتات الداتورة وبنسبة تثبيط بلغت 66.267 % مقارنة بمعاملة التركيز 50 ppm التي اعطت نسبة تثبيط بلغت 21.881 % ومعاملة السيطرة.