

Role of some Factors Affecting Crown Gall Disease Development

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The wounds, its depth and the time of inoculation of *Agrobacterium tumefaciens* on castor bean (*Ricinus communis*) plants affecting on the development of apricot crown gall disease whereas, the increase of wound depth and adding the pathogen after directly wounding plants increased the disease severity and gall formation. Where, the highest infection and gall formation resulted from inoculation of *A. tumefaciens* after directly wounding plants (zero time) at 3mm depth followed by 5mm depth, while the lowest gall formation and disease severity showed when adding *A. tumefaciens* suspension after 48 hrs. from wounding at one mm depth. On apricot plants (*Prunus armeniaca*), The plant hormones indole acetic acid (IAA) and cytokinin (CK) were decreased in the first stage of gall formation, while they were increased in the other stages of gall formation. Gibberellic acid (GA) was decreased in the first stage and then increased but it decreased again in the last stage.

On the other hand, abscisic acid (ABA) inactivation plant hormone showed an opposite result whereas, ABA was increased in the first stage of gall formation and then it was decreased in the other stages of disease. Also, *A. tumefaciens* isolates obtained from different hosts having different gall index (different in virulence) produced different rates of plant hormones IAA, GA, ABA and CK. But the rate of CK was found to be correlated with virulence. Also, crown gall disease development dependent on the production amount of the three plant hormones (IAA, GA3 and CK) and the ratio between them.

Fertilizers with (NPK) and compost resulted in decrease the severity of crown gall disease on apricot plants whereas; they reduced the number and weight of galls and root gall index in the treated plants compared with control. The best one treatment was (NPK) then (NP) and (NPK plus compost). Fertilizers with (NPK plus compost) due to increase the foliage of plants and in all fertilizer treatments increased the roots of plants compared with control except that treated with (NP). Also, no variation considered in the height of plants in all treatments, but the highest height recorded from (NPK plus compost) treatment. On the other hand, the uptake of (N) was increased in all fertilizers when used (N) alone or in combination with (P or K), also, (P) up take increased when used (P) alone or with (N or K). But (K) up take was increased only when used it with (N) or (P) and in combination with (NPK).

Keywords: Apricot, *Prunus* sect. *Armeniaca*, Crown-gall, *Agrobacterium tumefaciens*, disease severity, compost, NPK fertilizers, Indole acetic acid and Cytokinin.

Production of growth regulator, wounds, its depth, and mineral fertilizers, affecting the development of crown gall disease such as gall formation, disease severity and its effect on plant characters. So, studying these factors may help for effective control of the pathogen. Matveeva *et al.*, (2004) proposed that increasing the cytokinin / auxin ratio may lead to tumor formation on roots. Also, the development of plant tumors caused by *Agrobacterium tumefaciens* depends on the excessive production and synthesis of auxin (indole acetic acid) and cytokinin's by enzymes encoded on T-DNA genes integrated into the plant genome (Veselov *et al.*, 2003 and Liu and Nester, 2006).

On the other hand, the infection of *A. tumefaciens* was done when the bacteria attachment to the plant cells in the wound sites. So, avirulent *A. tumefaciens* bacteria failed to attach at wound sites of plants, as recorded by (Matthysse and McMahan, 2001, Tan *et al.*, 2005 and Li *et al.* 2008). Brencic *et al.* (2004 and 2005) who found that plant tumorigenesis by *A. tumefaciens* requires expression of the vir regulon in response to chemical signals that are thought to be released from plant wound sites.

With respect of the effect of manuring and fertilization on crown gall, Florenzano (1946) reported that nitrogen (N) fertilization increased disease severity while phosphorus (P) fertilization either alone or in combination with (N) reduced the number of galls. In 1975, Abd-El-Hafez *et al.*, found that (N) fertilization either alone or in combination with potassium (K) increased the severity of crown gall disease while (P) fertilization either alone or in combination with (N) showed considerable reduction in the number of galls. No difference between potassium treatment and the control in number of gall formation. Organic manuring treatment was found to give slightly higher number of galls per plant. Also, infected plants showed lower fresh and dry weight than healthy plants under all manuring treatments while the weight of root system was higher in diseased plants. The plant uptake of N, P and K was considerably lower in infected plants than healthy ones. The recent research use some microbial preparation or biopreparations such as Rhizoagrin containing (*Agrobacterium radiobacter*), Flavobacterin (*Flavobacterium* sp.) and Rhizoenterin (*Enterobacter aerogenes*) Pasyukov (2002), Zavalin *et al.*, (2003) and Gabibov (2006) as a source of fertilizers (N and P) and decreased the use of chemical compounds.

The aim of this study to know how some factors could (such as, wounding, growth regulators and fertilizers) effect on the development of crown gall disease on plants and this information may help to effect on these factors by some way due to decrease the development and severity of the disease (this way may be benefit to use in control the crown gall disease).

Materials and Methods

Preparation of Agrobacterium tumefaciens inoculum:

A suspension of *A. tumefaciens* isolate was prepared by inoculation of isolate No. Pp4 previously isolated from peach (previously identified by the authors Barakat *et al.*, 2009) in nutrient glucose broth (NGB) medium and incubated for 48
Egypt. J. Phytopathol., Vol. **39**, No. 1 (2011)

hrs at 28°C. The concentration of viable cells in the preparation was approximately adjusted to be 10^8 CFU/ml. Prepared suspension was used for inoculation in all experiments.

1- Effect of wounding:

Plants of castor-bean in pots 20 cm diam. (cv. Ahmar Malawi - 2-month-old) was used. Four replicates for each treatment were used and all plants were wounded by using tooth spikes (marking the depth by a ruler) in the crown region. The plants were divided into three groups depending on wound depth as follows: 1, 3 or 5 mm. Then, *A. tumefaciens* suspension was used to infest the soil at 3 different periods, after directly wounding plants (zero time), after 48 hrs. and 72 hrs. from wounding.

The results were recorded (by measured gall index, number and weight of galls, fresh and dry weight of root) after 45 days from the first inoculation (zero time) to the wounded plants. Whereas gall index (GI) as follows Abd-El- Aziz (2004):

$$GI = \frac{\text{Average diameter of gall}}{\text{Average diameter of stem}} \times 100$$

- Diameter of gall = diameter of stem with gall–diameter of stem only

Data in all experiments were subjected to statistical analysis using the completely randomized block design according to Snedecor and Cochran (1980). The means of treatment in this experiment were compared using the least significant difference (L.S.D).

- Design of the other experiments:

Apricot seedlings (Baladi cv., 4 months old) were used, and these plants were planted in pots (20 cm diam.- having 2 kg loamy soil). Four replicates were used for each treatment and all plants were wounded by using scalpel in the crown region.

2- Growth regulators production:

A. tumefaciens suspension isolate No. Ac1 previously isolated from almond (10^8 cfu/ml.) was added into the soil (50 ml / pot) having wounded plants. The growth regulators (indole acetic acid, gibberellic acid, abscisic acid and cytokinins) were determined and recorded in plants after 7, 15, 30 and 45 days from inoculation, respectively.

Also, suspension of different *A. tumefaciens* isolates from almond, peach, and rosella plants (these isolates previously identified by the authors Barakat *et al.*, 2009) were prepared in NGB medium and then the suspension of each isolates of *A. tumefaciens* was added into the soil at the rate of 50 ml/pot. The growth regulators were determined and estimation in plants after 48 days from inoculation (gall index of each infected plants were measured and recorded before estimation of growth regulators). In control, for growth regulators experiment plants wounded only without *A. tumefaciens* inoculation.

Measured of the hormones was carried out in Faculty of Agriculture Research Park (FARP), Cairo University. Then measured the ratio between the three plant hormones (Indole acetic acid, gibberellic acid and cytokinins)

Indole acetic acid, gibberellic acid, abscisic acid and cytokinins were extracted according to Prakash and Prathapasenan (1990). Two- gram samples of apricot root plants were homogenized in a mixture of methanol: chloroform (14:6 v/v) and maintained for one week at -20°C. The extracts were then filtered through a Whatman No. 5 filter paper and the residue was adjusted to pH 8.5 with 1N NaOH and transferred through a separating funnel to separate chloroform from methanol. The chloroform phase was discarded. The methanol phase was reduced to the aqueous phase in a rotary evaporator at 40°C. The aqueous phase was then adjusted to pH 2.5 with 1 NHCl and partitioned 3 times against ethyl acetate to take acidic free hormones and dried under vacuum at 40°C. The residue was dissolved in one ml of absolute methanol and transferred into an Eppendorf tube. The methanol was reduced to 100 µl.

A method for the simultaneous separation and determination of plant internal hormones in plants by gas chromatography with wide - pore capillary column was employed. The gas chromatographic conditions were as follows: FID detector; TR - 5MS capillary column, 30 m × 0.25 mm × 0.25 microns film. The injection temperature was, 270°C; the detector temperature, 270°C, carrier gas, 30 ml/min N₂. All components were separated in 20 min.

3- Effect of fertilizers:

The effects of nitrogen, phosphorus, and potassium fertilizers either alone or in combinations, together with organic manuring on the gall index as well as fresh and dry weight of apricot plants (Baladi cv., 4 months old) were studied. The suspension of *A. tumefaciens* isolate No. Pp4 (50 ml/pot) was added into the pots (20 cm diam.- having 2 kg loamy soil) containing the wounded plants.

Fertilizer treatments were applied by using ammonium nitrate (33 % N), calcium super phosphate (15 % P) and potassium sulphate (50 % K). These compounds were dissolved in water and used at the rate of 15, 33.3 and 10 g/l, respectively to have concentration of 0.5 % (this designer of requirements as recommended by Deciduous Fruits Department, Horticultural Research Institute, ARC. and according to Murat *et al.*, (2007).

Compost (Turned) produced from a mixture of animal manure and rice straw by ECARU Company was provided and analysis (Table 1) by Microbiology Department, Soil, Water and Environment Research Institute, ARC. and was applied at the rate of 50 g / pot.

Fertilizer treatments were applied as single (N, P, K, and compost) or as combinations (NP, NK, PK, NPK and NPK plus compost). After inoculation, the soil with *A. tumefaciens*, fertilizers (50 ml/pot) were added to the soil. Fertilizers were added to pots weekly for 42 days which equals 300 ml/pot for each treatment through the experiment. In control treatment soil was inoculated with *A. tumefaciens*

without fertilization. Disease readings were recorded after 45 days from inoculation by measured gall index, number of galls, fresh and dry weight of gall, fresh and dry weight of shoot and root and the height of plant.

Table (1): Physical and chemical analysis of compost.

Characters Weight of m ³ Kg	Compost 745
Humidity %	14
pH (1:10)	7.99
EC (1:10) (mm cm ⁻¹)	3.54
Ammonium nitrogen ppm	706
Nitrate nitrogen ppm	54
Total nitrogen %	0.98
Organic material %	24.57
Organic carbon %	14.25
C / N ratio	14.54 : 1
Total phosphorus %	0.74
Total potassium %	0.35

- Mineral content of treated plants:

Determination of total nitrogen, phosphorus and potassium were measured as adopted by Faculty of Agriculture Research Park

(FARP), Cairo University.

Plants were separated into galls, shoots and roots, and all tissues of the plant were immediately weighted. Then, separately plant was cut into small pieces and dried at 70°C for 24 hrs. in hot air oven. The samples of plants were ground into powder and used for determination of total nitrogen, phosphorus and potassium.

-Determination of total nitrogen:

Total nitrogen was determined by the usual Kjeldahl method as follows: The dried samples were digested with concentrated sulfuric acid in the presence of digestion catalysts (a mixture of copper sulfate and anhydrous sodium sulfate, 1:10). Then total nitrogen was determined titrimetric according to AOAC (1995).

-Determination of total phosphorus:

The phosphate ion reacts with molybdate to produce phospho-molybdate, which is finally reduced to a molybdenum blue. This compound was determined colorimetrically according to El-Merzabani *et al.* (1977) by using T80 uv/vis spectrometer from PG instruments LTd company with 650nm wavelength.

-Determination of total potassium:

This method provides for the acid digestion of the sample in a closed vessel device using temperature control microwave heating (180°C) for the metal determination by spectroscopic methods. The dried samples were digested in nitric acid by using Advanced Microwave digestion system ETHOS1 produced by

Egypt. J. Phytopathol., Vol. 39, No. 1 (2011)

Microwave equipment, Milestone ETHOS lab station, USA. The reagents were 7 ml of HNO₃ 65 %, 2 ml of H₂O₂ 30 %. Then, measured on Thermo Scientific iCAP 6000 Series of ICP emission spectrometer.

Statistical analysis:

The collected data were analyzed using ANOVA statistical analysis (Snedecor and Cochran, 1980). The least significant difference (L.S.D) was used to compare between the means.

Results

1- Effect of wounds, depth, and time of bacterial application:

Data in Table (2) revealed that a fresh wound is necessary for tumor cell formation. In general, the role of wounding is played within the first few days after the initial injury. Good tumor formation was evident on plants inoculated at 0 time. Determination of gall index (GI) which can be taken as a criterion of the severity of infection indicated the highest GI in plants inoculated at 0 time being 75, 259.3 and 228 at wound depth 1, 3 and 5 mm, respectively. The lowest figure was apparent following the 48 and 72 hrs. application of bacteria. All treatments and their statistically significant at 0.05 LSD.

Table (2): Effect of wounds on the incidence of gall development (GI) on castor bean plants.

Depth \ Time	Gall index %			
	1mm	3mm	5mm	Mean
0 time	75.0	259.3	228.0	187.4
48 hrs.	50.0	88.3	136.6	91.6
72 hrs.	80.0	80.0	150.0	103.3
Mean	68.3	142.5	171.5	128.8
LSD at 0.05				
Depth	43.11			
Time	86.22			
Depth × Time	49.78			

It is clear from the same table that not only the time of bacterial application effect on GI but also the injured cells expressed with different wound depths are the second factor on gall development. In this respect the size of the tumor is usually directly proportional to the severity of the wound (wound depth). It showed that the higher GI at wound depth 5mm and the lowest one at 1mm. This was statistically significant at LSD 0.05.

Data in Table (3) indicate that the eventual size of a tumor is independent on the number of bacteria in the inoculum used but the larger amount of wound sap present in massive wounds were related to the size of tumors which reflecting on the number of galls. It was obvious from these data that a significant difference among simultaneous inoculation with one mm wounds and the other deeper wounds (*i.e.*, 3

mm and 5 mm). No difference between 48 hrs. and 72 hrs. time of inoculation at all depth. This indicates that the wound sap produced or released from fresh and deep wounds have a significant important role in gall formation.

Table (3): Effect of wounds on the incidence of galls number on castor bean plants.

Depth \ Time	Av. No. of galls / plants			
	1mm	3mm	5mm	Mean
0 time	1.50	3.67	2.00	2.39
48 hrs.	1.00	1.00	1.30	1.10
72 hrs.	1.00	1.00	1.30	1.10
Mean	1.16	1.90	1.53	1.50
LSD at 0.05				
Depth			NS	
Time			NS	
Depth × Time			0.64	

NS: denotes non- significant

Data in Table (4) showed the fresh weight of galls which reflect on the number of gall formation. Data indicated that no significant differences at wound depth 1 and 5 mm at 48 and 72 hrs. but there are significant differences at depth 3 mm between 0 time which was 0.97 g and other time of inoculation 0.14 and 0.13 for 48 and 72 hrs., respectively.

Table (4): Effect of wounds, its depth and time of inoculation on the weight of galls developed on castor bean plants.

Depth \ Time	Av. No. of galls / plants			
	1mm	3mm	5mm	Mean
0 time	0.08	0.97	0.52	0.52
48 hrs.	0.05	0.14	0.53	0.24
72 hrs.	0.10	0.13	0.17	0.13
Mean	0.07	0.40	0.40	0.29
LSD at 0.05				
Depth			0.20	
Time			0.41	
Depth × Time			0.24	

It is clear from Tables (5) that no significant differences showed among all wound depths with different inoculation time. On the other hand, results of fresh weight of roots showed a significant difference in 3mm wound depth in inoculation time 72 hrs. compared with the other times of inoculation (0 time and 48hrs), as well as the same at 5mm depth after 72 hrs. from wounded plants. With respect of dry weight of root, the same figure was observed.

Table (5): Effect of wound depth and time of inoculation on the roots fresh and dry weight of castor bean plants.

Depth Time	Av. Wt. of fresh and dry roots / plants (g)								
	1mm		3mm		5mm		Mean		
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	
0 time	9.95	2.17	4.19	1.37	7.45	1.74	7.20	1.76	
48 hrs.	4.81	1.19	7.72	2.10	6.84	1.57	6.46	1.62	
72 hrs.	6.74	1.54	13.61	3.22	2.79	0.69	7.71	1.82	
Mean	7.17	1.63	8.51	2.23	5.69	1.33	7.12	1.73	
LSD 0.05		For fresh wt.				For dry wt.			
Depth		NS				NS			
Time		5.71				1.14			
Depth x Time		NS				NS			

NS: denotes non- significant

2- Growth regulators production

Data in Table (6) indicate that different isolates of *A. tumefaciens* isolated from different hosts could produce different rates of growth regulators in infected apricot plants. Almond isolate which causes a high root gall index (i. e. GI 200) produced the highest rates of indole acetic acid (IAA) 1.69 μg / 100g fresh root weight (FW) followed by the rosella isolate having GI of 125 which produced 0.97 μg / 100g FW. However, the more aggressive isolate of peach (Pp4) having GI of 250 produced 0.084 μg / 100g FW. Whereas, gibberellic acid (GA3) production was different compared with IAA, the lowest rate from almond isolate (0.39 μg /100g FW) then rosella isolate (1.88 μg /100g FW) and the highest rate production from peach isolate (2.39 μg /100g FW).

Table (6): Effect of different *Agrobacterium tumefaciens* isolates on growth regulators production (μg /100g fresh weight FW).

Substances isolates	Source of isolates	Gall index	Growth regulators / plants (μg / 100 g F.W)			
			IAA	GA3	ABA	CK
Ac1	Almond	200	1.690	0.39	0.015	871.33
Pp4	Peach	250	0.084	2.39	0.059	1431.65
Hs19	Rosella	125	0.970	1.88	0.068	579.68
Control	N	0	4.77	2.74	0.07	494.37

IAA = Indole acetic acid; GA3 = Gibberellic acid; ABA = Absciscic acid; CK = Cytokinin; N = Non infected plants

Production of absciscic acid (ABA) was highest by rosella isolate (0.068 μg /100g FW) followed by peach isolate (0.059 μg /100g FW) and the lowest one was produced by almond isolate (0.015 μg /100g FW). But production rate of cytokinin

(CK) have a variation according to the isolates aggressiveness on apricot plants whereas, peach isolate (has the highest GI) showed the highest rate of cytokinin's production (1431.65 $\mu\text{g} / 100\text{g FW}$) followed by almond isolate in which cytokinin was 871.33 $\mu\text{g} / 100\text{g FW}$ and the last one was rosella isolate which produced the lowest rate of cytokinin (579.68 $\mu\text{g} / 100\text{g FW}$).

Data in Table (7) indicate that the production and ratio of IAA, GA3 and CK were affected the crown gall disease development. Although, the highest GI produced from peach isolate (Pp4), this isolate has the highest CK / IAA and CK / IAA with GA3 ratio but moderate in CK / GA3 ratio. Isolate (Ac1) from almond have moderate GI have the lowest CK / IAA ratio, highest CK / GA3 ratio and moderate CK / IAA with GA3 ratio. The lowest GI produce by rosella isolate (Hs19) which have moderate CK / IAA ratio and the lowest CK / GA3 and CK / IAA with GA3 ratio.

Also, Table (7) prove that the highest correlation between the isolate's aggressiveness and the growth regulators production ratio was recorded between CK / IAA with GA3 ratio which was 0.97, followed by CK / GA3 ratio (0.88) and the last and lowest one was CK / IAA ratio (0.67).

Data indicate that also production of GA3 was necessary to crown gall disease development. So, gall formation development dependent the production of the three plant hormones together (IAA, GA3 and CK) and the ratio between CK / IAA with GA3, not depended on the production of IAA and CK as well as the ratio between them. Whereas the highly aggressiveness isolate have the highest CK / IAA with GA3 ratio and the lowest isolate in aggressive produced the lowest CK / IAA with GA3 ratio.

Table (7): Differentiation between growth regulators ratio in apricot plants infected with different isolates of *Agrobacterium tumefaciens*.

Isolates	GI	CK / IAA ratio	CK / GA3 ratio	CK / IAA + GA3 ratio
Ac1	200	515.58 : 1	739.20 : 1	418.91 : 1
Pp4	250	17043.35 : 1	599.02 : 1	578.68 : 1
Hs19	125	596.91 : 1	308.34 : 1	203.40 : 1
Control	0	103.64 : 1	180.43 : 1	65.83 : 1
Correlation (r)	-	0.67	0.88	0.97

IAA = Indole acetic acid; GA3 = Gibberellic acid; ABA = Abscisic acid; CK = Cytokinin; Control = Non infected plants

Data in Table (8) and Figs. (1, 2 and 3) showed that production of growth regulators was different according to development of gall formation. Whereas production of IAA was decreased in plants infected with *A. tumefaciens* from 0.84

$\mu\text{g} / 100\text{g FW}$ to $0.48 \mu\text{g} / 100\text{g FW}$ after 15 days from inoculation compared with the control. At the same time, it was increased from 0.91 to $1.59 \mu\text{g} / 100\text{g FW}$, then IAA was increased again in the infected plants to $0.83 \mu\text{g} / 100\text{g FW}$ after 30 days from inoculation and increased again in the last stage of gall formation after 45 days from inoculation to $1.69 \mu\text{g} / 100\text{g FW}$ compared with the control. After 30 and 45 days IAA increased to 1.82 and $4.77 \mu\text{g} / 100\text{g FW}$, respectively.

Also, production of gibberellic acid (GA3) was decreased in infected plants from $17.30 \mu\text{g} / 100\text{g FW}$ to $10.58 \mu\text{g} / 100\text{g FW}$ after 15 days from inoculation but in control the production of GA3 was increased from 2.57 to 7.06 at the same time. Production of GA3 in the roots of infected plants was increased again to $10.75 \mu\text{g} / 100\text{g FW}$ after 30 days from inoculation then decreased in the last stage of gall formation reaching to $0.39 \mu\text{g} / 100\text{g FW}$ after 45 days from inoculation. In non-infected plants, GA3 increased to $29.84 \mu\text{g} / 100\text{g FW}$ and then decreased to $2.74 \mu\text{g} / 100\text{g FW}$ at the same time.

But, the production of abscisic acid (ABA) showed different trend as it increased from $0.75 \mu\text{g} / 100\text{g FW}$ to $1.03 \mu\text{g} / 100\text{g FW}$ after 15 days from inoculation and then sharply decreased more at the last stage of gall formation to $0.015 \mu\text{g} / 100\text{g FW}$. In control check plants (ABA) was increased from 0.15 to $0.29 \mu\text{g} / 100\text{g FW}$ and increased to $1.83 \mu\text{g} / 100\text{g FW}$ then decreased to $0.07 \mu\text{g} / 100\text{g FW}$ at the respective periods.

On the other hand, initial cytokinins (CK) content of apricot plants was decreased after inoculation within 15 days to reach $24.65\text{mg} / 100\text{g FW}$, then increased to $42.82 \text{mg} / 100\text{g FW}$ after 30 days and at the last stage of gall formation after 45 days it remarkably increased to reach $871.33 \text{mg} / 100\text{g FW}$ compared with $494.37 \mu\text{g} / \text{g FW}$ in control check plants at the same time. So, production of cytokinins in the infected plants by *A. tumefaciens* in all stage of gall formation showed an increment compared with the control plants.

Table (8): Differentiation of growth regulators production in different stages of gall formation by *Agrobacterium tumefaciens* (Ac1 isolate) in apricot roots.

Stages	GI	Growth regulators amount ($\mu\text{g} / 100 \text{g}$ root fresh weight)							
		IAA		GA3		ABA		CK	
		Ac1	(C)	Ac1	(C)	Ac1	(C)	Ac1	(C)
7 days	0	0.84	0.91	17.30	2.57	0.750	0.15	52.83	10.85
15 days	44	0.48	1.59	10.58	7.06	1.030	0.29	24.65	9.36
30 days	107	0.83	1.82	10.75	29.84	0.290	1.83	42.82	2.85
45 days	200	1.69	4.77	0.39	2.74	0.015	0.07	871.33	494.37

(C): Control non infected plants

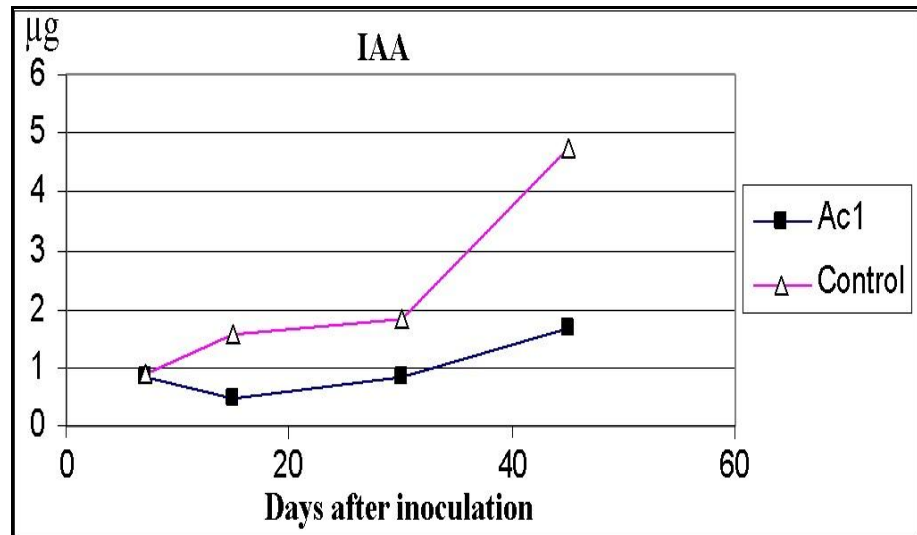


Fig. (1): Differentiation of production of IAA growth regulators ($\mu\text{g} / 100\text{g}$ fresh weight FW) in different stages of gall formation by *Agrobacterium tumefaciens* in apricot roots. Ac1: Almond isolate Control: Non infected plants

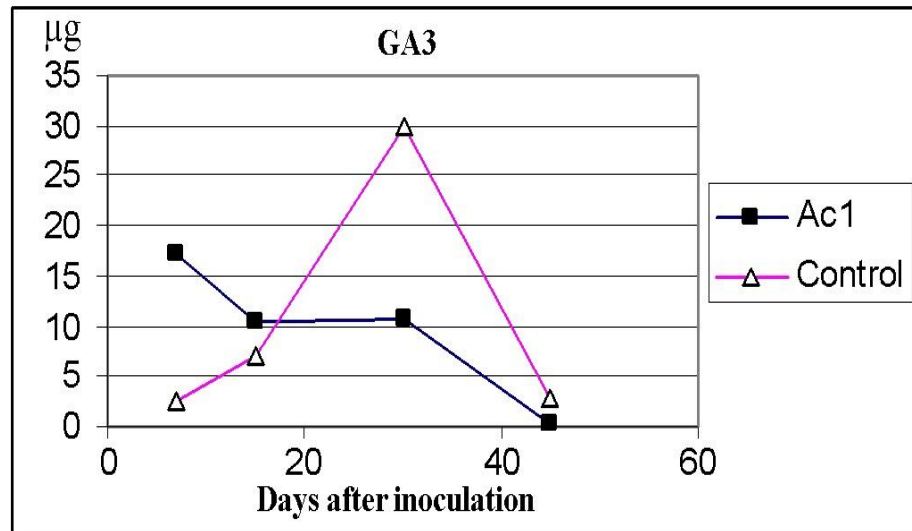


Fig. (2): Differentiation of production of GA3 growth regulators ($\mu\text{g} / 100\text{g}$ fresh weight FW) in different stage of gall formation by *Agrobacterium tumefaciens* in apricot roots. (Ac1: Almond isolate; Control: Non infected plants)

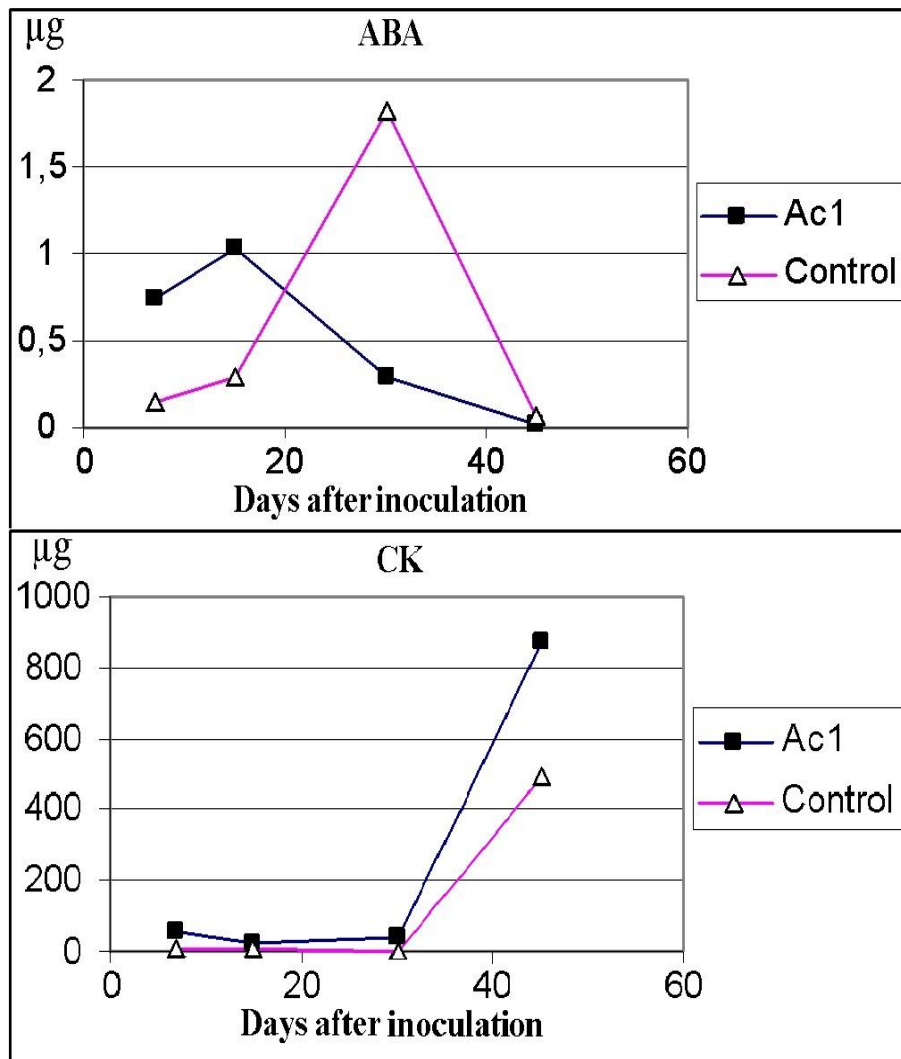


Fig. (3): Differentiation of production of ABA and CK growth regulators ($\mu\text{g} / 100\text{g}$ fresh weight FW) in different stage of gall formation by *A. tumefaciens* in apricot roots. (Ac1: Almond isolate; Control: Non infected plants)

a- Effect of fertilization on gall formation

It is clear from Table (9) that the average number of galls was 3.5 /plant in the control treatment. Phosphorus and potassium fertilization either alone or in combination with nitrogen resulted in a considerable reduction in the average number of galls reaching 1.7, 1.3, 1.3, 1.0 and 1.0 for P, K, NP, PK and NPK treatments, respectively.

Although nitrogen fertilizer alone or in combination with compost (produced from a mixture of animal manure and rice straw) was found increase of average of gall formation than the other treatment of NP and NK. It seems that the presence of two fertilizers in combination resulted in balanced absorption of two elements. Such conditions might give the growing plants higher resistance to the disease.

Table (9): Effect of fertilization on number of galls, gall index and the weight of galls caused by crown gall disease on apricot.

Treatments	Av. No. of galls / plant	Gall index (GI)	Av. wt. of galls / plant (g)	
			Fresh	Dry
N	2.0	158.8	0.33	0.10
P	1.7	183.3	0.10	0.04
K	1.3	124.8	0.18	0.06
NP	1.0	99.8	0.10	0.04
NK	1.0	112.3	0.19	0.09
PK	1.3	191.5	0.14	0.08
NPK	1.0	87.3	0.22	0.09
Compost (C)	2.0	174.5	0.11	0.03
NPK+ (C)	1.8	143.8	0.39	0.10
Control	3.5	191.3	0.18	0.07
LSD% at 0.05	NS	1.1	NS	NS

With respect to the fresh and dry weight of galls, it was found that although control treatment showed higher incidence of galls than other treatments alone or in combination, it showed lower fresh and dry weight of galls. This indicates that galls formed on the control treatment were smaller in size. The highest fresh and dry weights of galls, however, observed in N alone and NPK and NPK plus manure reaching 0.33, 0.22 and 0.39g / plant. The lowest figure was observed in P and K alone or in combination (PK) and in combination with nitrogen (NP) and (NK).

It is clear from Table (10) that manuring treatments showed significant differences than the control. Nitrogen application in combination with phosphorus slightly decreased fresh and dry weights, while both NPK and compost treatments resulted in considerably increase in plant weight over the other treatments. It was found from the same Table that there is clear relation between the plant height and the vegetative growth of apricot plants. This might be a reflection of the effect of the disease of plant growth especially in applications of (N), (NP) and compost treatments whereas the plant height reaching 39.7, 34.5 and 36.7 cm, respectively.

Table (10): Effect of fertilization on fresh and dry weight of shoot, roots, and height of apricot plants

Treatments	Av. wt. of shoot / plant (g)		Av. wt. of root / plant (g)		Av. Height of plant (cm)
	Fresh	Dry	Fresh	Dry	
N	2.70	1.03	2.08	0.94	39.75
P	3.14	1.11	1.52	0.60	43.33
K	3.05	1.06	1.88	0.77	44.25
NP	1.22	0.76	1.09	0.36	34.50
NK	3.00	1.34	2.12	0.79	42.50
PK	3.96	1.31	1.50	0.57	45.25
NPK	3.11	1.15	2.18	0.84	41.00
Compost (C)	4.02	1.39	1.70	0.63	36.75
NPK+ (C)	4.49	1.61	2.69	1.20	45.00
Control	4.66	1.46	1.35	0.52	44.75
LSD %0.05	1.7	NS	0.915	0.45	NS

Data in Table (11) showed that nitrogen percentage of the control was considerably lower than other treatments. It seems that total nitrogen per plant could be taken as a good criterion for plant uptake rather than determining its percentage. Thus, it is clear that total nitrogen percent was high in treatment receiving N, NPK and NPK plus compost. Total N per 100 g plant reached 3.9, 2.8 and 3.0g for these treatments, respectively. The same figure was also observed for K and P treatments. The lowest N uptake was observed in NP treatment.

With respect to plant uptake of phosphorus as indicated by total phosphorus per plant it is clear that the uptake was similar between all application treatments and the control. It was found from such data that NPK treatment and inoculation influenced with K content of plants. Total K uptake was higher compared with other treatments, this highest uptake in NPK treatment which indeed showed a low disease severity as indicated by gall index (GI).

Table (11). Effect of fertilization on mineral composition of apricot plants.

Fertilizers Treatment	mineral nitrogen (g / 100g)	content phosphorus (g / 100g)	/ plant potassium (mg / 100g)
N	3.967	0.18	4.456
P	2.661	0.22	3.673
K	2.892	0.19	4.982
NP	1.681	0.28	4.262
NK	2.871	0.22	6.306
PK	2.295	0.25	6.111
NPK	2.891	0.26	6.776
Compost (C)	2.398	0.26	5.451
NPK+ (C)	3.003	0.28	4.717
Control	0.503	0.23	5.451

Discussion

The purpose of this study to know how some factors could (such as, wounding, growth regulators and fertilizers) effect on the development of crown gall disease and increased or decreased its severity on plants. This information may help to effect on these factors by some way to decrease the development and severity of the disease (these ways may be benefitted to use in control the crown gall disease).

In crown gall disease wound site are necessary step in the infection to induce gall formation. The attached bacterium initiates the transfer of T- DNA under the influence of chemicals produced by wounded plant cells, it is clear that if wounds didn't find, the bacterium couldn't attach to the host cell (Tan *et al.*, 2005 and Li *et al.*, 2008).

Also, wounding is known to be essential for crown gall infection due to wound-released chemicals that induce the expression of virulence genes in *A. tumefaciens* leading to transfer of T-DNA to the plant cell. In this respect, many investigations belong to wound sap are of considerable interest (Wise *et al.*, 2005 and Brencic *et al.*, 2004 and 2005).

The eventual size of tumor is independent of the number of bacteria comprising an inoculum; the size of the tumor is directly proportional to the severity of the wound. In the present study, the wound depth of 3 and 5 mm resulted an increase in severity of disease while less size of galls was shown at wound depth 1mm due to the higher products released from deeper wounded tissues.

In addition, time of inoculation with *A. tumefaciens* after wounding plant was necessary for successful infection. It was proved that inoculation directly after wounding plants caused an increase in its severity and in gall formation compared with long periods after wounding 48 hrs. and 72 hrs. of inoculation in which wound healing processes started fast in superficial wounds compared with deeper. The present results agree with those obtained by Hildebrand 1942, Braun 1952 and Brencic *et al.*, 2004 and 2005.

Growth regulators production by isolates of *A. tumefaciens* revealed different amounts and ratio from IAA, GA3, ABA and CK in the plants and no relationship between the production amount of IAA, GA3 and ABA individually and the aggressiveness of isolates but production amount of CK only and IAA with GA3 was increased when the isolates aggressiveness was high. Whereas the highest GI obtained from peach isolate and the highest amount and ratio of CK and IAA with GA3 produced from this isolate. While the lowest isolates in aggressive (GI 125) produced the lowest amount and ratio of CK and IAA with GA3.

So, results revealed that *A. tumefaciens* aggressiveness dependent the production amount of the three plant hormones IAA, GA3 and CK together. Since some similarity articles recorded that GA3 was tested for its effect on tumor induction by *A. tumefaciens* in primary pinto bean (*Phaseolus vulgaris* L) (Anand *et al.*, 1975), also, ElKhalifa and Yousif (1974) recorded GA3 was found to increase secondary

Egypt. J. Phytopathol., Vol. 39, No. 1 (2011)

tumor initiation by over 100% in castor bean infected with *A. tumefaciens* and the mechanism of secondary tumor initiation is similar to that of primary tumor initiation.

On the other hand, IAA, GA3, ABA and CK showed differentiation in production amount through gall formation development. In transformation period (transformed the normal cell to primary tumor cell through 7 days from inoculation) activators growth regulators IAA, GA3 and CK were decreased but inhibitor growth regulators ABA was increased.

In the duplication period (primary tumor cell increased and transformed to tumor mass after 15 days from inoculation) activator growth regulators were increased, and inhibitor was decreased at this stage up to 30 days from inoculation.

The organization and differentiation period (the crown gall tumor after 45 days from inoculation) the IAA and CK were continuous increased but GA3 and ABA decreased. Also, CK was increased in the infected plants at all stages compared with the control. These periods of crown gall development and formation were agreement with the recorded by Klein (1962).

Results obtained improved that the production of IAA and CK and the imbalance in their ratio were leading to the development of tumors and gall formation. Also, cytokinins play a crucial role in the tumor development. These results as previously recorded by many investigators (Matveeva *et al.*, 2004, Liu and Nester 2006, Alekseeva *et al.*, 2008 and Kamada-Nobusada and Sakakibara 2009). Also, GA3 ratio play a role in *A. tumefaciens* aggressiveness but through gall information whereas, it was increased in the first period then decreased. These results were recorded also by Gong and Gao (2008).

It is well known that balanced fertilization is one of the important means for controlling plant diseases. In this study, the effect of N, P and K fertilization either alone or in combination on the severity of crown gall disease, plant growth and plant uptake of nutrients was investigated. Generally, mineral fertilizer treatments in the plants decreased the severity and development of the crown gall disease. Thus, they reduced the number of galls compared with control and also gall index (GI) which can be determine and taken as a criterion of the severity of infection. Potassium fertilizer alone or in combination with nitrogen resulted in the higher reduction in the number of galls. Gall index (GI) varied with some fertilizers or their combinations such as (K), (NP) and (KP) compared with control receiving no fertilizers. The lowest GI was in a balanced fertilizer treated plants with (NPK) followed by combination. No significant results were noticed in the weight of galls in all fertilizer treatments. From these results, it can be indicated that fertilizer treatments have decreased the number of galls per plant even though the unfertilized plants have a high gall formation. So, mineral fertilization especially in combination of (NPK and NP) could decrease gall formation on the infected plants compared with control. A similar result was reported by Florenzano (1946) and Hussin and Deep (1965). Nitrogen fertilizer, increased the susceptibility of plants to crown gall, was evidenced by the high incidence of gall formation. This was because of nitrogen

in increasing the vegetative growth and rendering the tissue succulent increasing disease severity, which was explained by Abd-El-Hafez *et al.*, (1975), who reported a substantial decrease in fresh and dry weight of shoots in infected plants treated with (N) and (NP). These data are the same of our results. The highest fresh and dry shoots resulted in the fertilizer combination NPK and compost treatments.

The fresh and dry weight of roots showed significant differences than the control, nitrogen in combination with phosphorus (NP) application decreased fresh and dry weigh of roots and the highest one resulted from NPK with compost fertilizers compared with the control.

The effect of fertilizers and inoculation with *A. tumefaciens* on mineral composition of plants showed that all fertilization increased the N uptake in plants compared with the control. The highest one was recorded in (N) and (NPK with compost) treatments. As well as P fertilizer showed an increase in uptake recorded in the combination fertilizers (NPK with compost) compared with control while it was decrease in plants treated with (P) alone. The plants treated with (K) showed highest uptake in (NPK) combination fertilizer while it was decrease in treatment with K alone.

Conclusion

These results proved that combination between fertilizers (NPK with compost) has increased plant growth which may be due to the decrease in disease severity and gall development. These results are in accordance with phase of Zavalin *et al.*, (2003).

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دور بعض العوامل المؤثرة على تطور مرض التدرن التاجي

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الجروح و اختلاف اعماقها و ميعاد اجراء التلقيح بميكروب التدرن التاجي في النبات يؤثر على تطور و شدة المرض حيث وجد انه في نباتات الخروج كلما زاد عمق الجروح مع اضافة المسبب المرضي عقب التجريح مباشرة كلما زادت شدة الاصابة، حيث سجلت اقل شدة اصابة بالمرض و قلة تكوين الاورام عندما تم اضافة الميكروب بعد 48 ساعة من اجراء عملية التجريح و ذلك عندما كان الجرح على عمق 1 ملم و سجلت اعلى شدة للاصابة و تكوين الاورام عند اضافة الميكروب عقب اجراء عملية التجريح مباشرة على عمق 3 ملم يليها على عمق 5 ملم.

في نبات المشمش وجد ان الهرمونات النباتية حمض اندول اسيتيك و السيتوكينين يحدث انخفاض في كميتهما في اول مراحل الاصابة بمرض التدرن التاجي ثم تزدادان بعد ذلك حتى تمام اكتمال تكوين الورم اما حمض الجبريلليك فتتخفص كميته في اول المراحل ثم يزداد ثم ينخفض مرة اخرى عند تمام اكتمال تكوين الورم، و من ناحية اخرى نجد ان الهرمون النباتي المثبط للنمو حمض الابسيسيك يظهر عكس ما سبق حيث تزداد كميته في بداية تكون الورم ثم يحدث لكميته انخفاض في المراحل التالية حتى تمام اكتمال تكوين الورم.

ايضا تختلف كمية منظمات النمو المختلفة سواء منشطة للنمو او مثبطة له (حمض اندول اسيتيك، السيتوكينين، حمض الجبريلليك وحمض الابسيسيك) و المنتجة داخل النبات نتيجة الاصابة بعزلات الاجروباكتيريوم تيوميغاشنس المختلفة في الشدة المرضية و المعزولة من عدة عوائل مختلفة، و لكن وجد ان كمية السيتوكينين المنتجة في النباتات المحقونة تتناسب طرديا مع الشدة المرضية للعزلة التي تم حقنها. ايضا تطور تكوين الورم و زيادة الشدة المرضية للعزلات يرجع للكمية المنتجة من منظمات النمو الثلاثة مجتمعة (حمض اندول اسيتيك، السيتوكينين وحمض الجبريلليك) و النسبة بينهم.

ادى استخدام التسميد المتكامل باستخدام النيتروجين، الفسفور و البوتاسيوم (NPK) مع الكمبوست الى تقليل شدة حدوث المرض على نباتات المشمش حيث ادى لخفض عدد الاورام المتكونة و اوزانها و قلة افطارها للنباتات المسمدة مقارنة بمعاملة المقارنة، كما ادى التسميد المتكامل الى زيادة المجموع الخضري للنباتات في حين حدث انخفاض في المجموع الخضري عند التسميد بالنيتروجين بمفرده او مع الفوسفور، و ارتفع و زاد المجموع الجذري للنباتات التي تم تسميدها في جميع المعاملات السمادية المستخدمة خاصة في معاملة التسميد المتكامل، و لم يلاحظ وجود اختلافات واضحة في اطوال النباتات نتيجة التسميد. وقد ارتفع محتوى النباتات من النيتروجين و الفوسفور الكلى في جميع المعاملات السمادية و لكن محتوى النباتات من البوتاسيوم لم ترتفع الا عند اضافته مع النيتروجين او مع الفوسفور و عند اضافة الثلاثة معا (NPK) فقط.