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Characterization and Biocontrol of Crown Gall Disease in Jordan

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ABSTRACT

Agrobacterium tumefaciens, the causal agent of crown gall, was isolated from peach, almond, apricot, cherry, nectarine, plum, apple, pear, *cichorium pupumilum*, olive, grapevine, carob, pomegranate and roses. 200 pathogenic isolates were separated into biovars based on biochemical tests. Biovar 1 (60%) and biovar 2 (23.5%) were found to be the most common isolates and isolated mainly from stone fruits in addition to olive, carob, pomegranate, and only biotype 3 isolates were isolated from grapes. Only (70.5%) of the isolates were found to be sensitive to agrocin, where the majority of them belonged to biovar 1. However, 15% of the isolates were found to be intermediate and didn't belong to any of the biotypes.

The different tested bioagents and garlic extract were found to be effective in inhibiting the growth of three tested tumorgenic strains (Jordanian isolate 186, C58 and B6 agrocin sensitive), under laboratory condition , as well as in reducing the percentage of galled tomato or GF677 seedlings.

KEYWORDS: *Agrobacterium tumefaciens*, crown gall, stone-fruits, agrocin, biotype, bioagents, garlic extract, tomato, GF677.

1. INTRODUCTION

Agrobacterium tumefaciens (Smith and Townsend Conn.) the causal agent of crown gall disease is considered a soil-borne pathogen, distributed world-wide including the Mediterranean countries (Raabe, 1964; Al-Karablieh and Khlaif, 2002), with world- wide host range, the majority of those are dicotyledonus plants including stone fruits, pome fruits, grapevine and pomegranate. Farmers' and nurseries' growers may suffer serious economic losses as galled plants show growth reduction, decline, and are unmarketable which have to be discarded. In the USA, the losses due to crown gall were assessed to a total of 23 million dollars (Kenndey and Alcorn, 1980).

Different control measures have been used against crown gall, among them is the dipping of rooted plants into chemicals and antibiotics which have given incomplete crown gall control and often phytotoxic (Grim and Sule, 1981) and most of the seedlings could not be used for propagation (Grim, 1987). Furthermore, soil fumigation gave incomplete control of crown gall (Pu and Goodman, 1993) and was reported to induce an unexpected increase in disease incidence (Deep *et al.*, 1968; Riao *et al.*, 1997), soil solarization reduced the population of pathogenic *Agrobacterium,* but after a period of time, non-pathogenic strains may conjugate with pathogenic ones (Raio *et al*., 1997). Biological control by pre-planting dip in a suspension of *Agrobacterium radiobacter* K84 (New and Kerr, 1972), has been successful in different regions of the world including the Mediterranean countries (Bazzi and Mazzuchi, 1978; Tawfiik and Abd-El-Moity, 1986; Lopez *et al.*, 1987; Bouzar *et al*., 1991; Farker and Hass, 1985; Fakhori and Khlaif, 1996; Ramon *et al.,* 2000). However, natural *A. tumefaciens* population that resist

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K84 treatment are known to exist and K84 is not effective on infected asymptomatic plants ineffective on certain tested strains and biovars and on certain crops as in apple nurseries (Moore, 1979; Grim and Sule, 1981; Grim and Vogelsanger, 1983; Zoina and Raio, 1999).

Due to these difficulties in crown gall control, there is a need for a safe reliable and friendly environmental method for the elimination or reduction of *A. tumefaciens* in soil. This study was conducted to test the effect of some bioagents other than K84 on the growth of *A. tumefaciens* in the lab as well as on crown gall development in the nurseries.

2. MATERIALS AND METHODS

Isolation from Various Plants

Samples of newly developed galls from trees or seedlings suspected to be as a result of crown gall infection were collected from the different trees' growing areas in Jordan.

Tumors were washed under tap water, surface disinfected by dipping them into sodium hydrochlorite solution, 1% for 10-20 minutes according to Moore (1988) and Schaad *et al*. (2001) and were rinsed with sterile distilled water, plotted onto sterile filter paper to dry then the outer layer was removed with sterile scalpel, small pieces were aseptically removed from each tumor, placed into few drops of sterile distilled water, then the resulted suspension was left to stand for 30 minutes and a loopfull of the resulted suspension was streaked on the surface of a dried plate of D1 medium modified by Kado and Heskett (1970) according to the method described by Moore *et al*. (1988).

Inoculated plates were incubated at 25± 2◦C till bacterial growth developed dark green olive colonies showing *Agrobacterium* colony characteristics were selected, purified by preparing a suspension of the colonies in sterile distilled water and restreaked on KB medium, colonies fluorescent under ultraviolet light were discarded. The obtained bacterial isolates were grown on NA slants kept in a refrigerator for further identification.

Identification and Biotyping

Twenty four hour old cultures of the obtained isolates were subjected to urease production and esculin hydrolysis tests, to test for the possibility of *Agrobacterium* isolation, (Moore et al., 1988). The isolates proved to be *Agrobacterium* based on the results of their reactions to these tests, then they were subjected to biochemical and physiological, tests to divide them into biotypes as described by Moore *et al*. (1988) and Schaad *et al*. (2001). The tests included: oxidase, 3-ketolactose production; alkali production from L-tartaric and propionic acids; acid production from: sucrose, melezitose and erythritol, action on litmus milk; 2% sodium chloride tolerance, pigmentation on ferric ammonium citrate; growth on simmons citrate medium and agrocin sensitivity.

Pathogenicity Test

Four one month old seedlings of tomato (*Lycopersicon esculuntum* cv. Maramand) and Kalanchoe (*Kalanchoe daogemontiana*) were used as indicator plants, the seedlings were wounded forming a slit in the crown area by a sterile scalpel then a mass of 24 hour bacterial culture was applied to the wounded area with a sterile tooth pick. Also, tomato seedlings were inoculated with sterile distilled water, and another set was inoculated with known pathogenic *Agrobacterium tumefaciens* isolate to serve as control. Inoculated seedlings were kept on a greenhouse bench at 25± 2C^{o}, checked periodically for tumor formation (Al-karablieh and Khlaif, 2002).

All the above mentioned tests were run against the reference cultures of *Agrobacterium tumefaciens* C58 and B6 (biovar 1); *A.rhizogense* 8302 (biovar 2), and *Agrobacterium vitis* 5858 (biovar 3). These reference cultures were provided by M.Lopez IVIA Valancia, Spain.

Sensitivity of *Agrobacterium* Isolates to Agrocin K84

Because biological control of crown gall is highly correlated with agrocin sensitivity of the pathogen, the obtained isolates were tested for agrocin sensitivity on MG agar plates as described by Stonier (1960) modified

by Moore *et al*. (1988). The diameters of inhibition zones were measured after 3 days of inoculation. Agrocin 84 sensitive strain C58 tumorgenic biovar 1 and the agrocin 84 resistant strain B6 tumorgenic biovar 1 were used as control.

Screening of Bioagents

The effect of the filter sterilized extracts of *Bacillus subtilis*, *Penicillium* sp, *Trichoderma harzianum*, K84 and K1026 (provided by M.Lpoez IVI A, Valenciia, Spain) in addition to garlic extract were tested as bioagents on the growth of the *Agrobacterium tumefaciens* B6, C85 and the Jordanian isolate 186, in plates as well as on tumor developing on tomato and GF677 seedling roots artificially inoculated with the *Agrobacterium* isolates suspension (Cooksey and Moore, 1980).

a- Laboratory Sensitivity Test

The different bioagents were grown on nutrient broth placed onto a shaker at room temperature for 3 days, and then filter sterilized; garlic extract (obtained by grinding 1 gram / 10ml sterile distilled water) with a mortar and pestle. Three wells were cut into the surface of PDA plate whose surface was already inoculated with one of the tested bacterial isolates (C58, B6 and the Jordanian isolate 186). The wells were filled with the extract of one of the three tested bioagents; each treatment was replicated four times then plates were incubated at 25 ± 2^{o}C for three days, checked for inhibition zone formation around the wells, then the diameters of the inhibition zones were measured and the average diameter of the inhibition zones for the four replicates for each treatment was calculated.

b- Preliminary Study on Tomato Seedlings

The effective bioagents extract for the inhibition of tumorgenic *Agrobacterium* growth *in vitro* were chosen to test their effect on tumor developing on the roots of tomato seedlings artificially inoculated with the bacterial suspension.

The roots of four-week-old tomato seedlings, cv. Maramand washed with sterile distilled water, dipped separately into the extract of one of the tested bioagents for 30 minutes, then the seedlings were planted separately into (15 × 15 cm) cylindrical pots filled with Methyl Bromide (MBr) fumigated soil mixed with sand and peatmoss, each seedling was planted in one pot. Two days later, the pots were watered with 150 ml of 10^7 CFU/ml bacterial suspension of one of the tested isolates (C58, B6 and the Jordanian isolate 186). The roots of another set were watered with bacterial isolate suspensions only to serve as control. Each treatment consisted of 20 seedlings and replicated 4 times, the treated seedlings were kept on a green house bench in a complete randomized block design. Three months later, the seedlings were uprooted and examined carefully for tumors on their roots.

c- GF677

The same procedure described previously for tomato seedlings was followed using one-year-old GF677 (*Prunus persica* x *Pruns amygdalus*) seedlings, imported from France, where the roots of GF677 seedlings were dipped separately for 30 mins in one of the tested bioagents, planted separately into cylindrical pots (20 × 20 cm) filled with MBr fumigated soil mixed with sand and peatmoss. Two days later, the soil surface of each planted pot was watered with 250 ml of 10^7 CFU/ml bacterial suspension of one of the tested isolates (C58, B6 and the Jordanian isolate 186). Pots were placed on green house bench in a complete randomized block design, and checked periodically for tumors on the crown area.

Forty seedlings were used for each treatment, other 40 seedlings of GF677 were dipped into 10^7 CFU/ml bacterial suspension of each of the tested *Agrobacterium* isolates to serve as control. The tested seedlings were uprooted 9 months later and were checked carefully for developing tumors on their root at the end of each experiment period.

The different treatments were evaluated based on disease incidence. Disease incidence was determined as the percentage of infected seedlings out of the total artificially inoculated seedlings in each replicate. Each

seedling which shows just one tumor on its roots was considered to be infected. Then the average percentage of each treatment was calculated (Khlaif, 2004).

3. STATISTICAL ANALYSIS

Data on the disease incidence were statistically analyzed and the significant means were separated by LSD test.

4. RESULTS

Characterization and Biotyping:

Two hundred isolates expected to be *Agrobacterium* based on their positive reaction to esculine hydrolysis and urease production tests, induced overgrowths on either tomato or *Kalanchoe* or both and were identified as *Agrobacterium tumefaciens.*

Based on the reaction of these isolates to the different biochemical and physiological tests, the isolates could be grouped into the following groups (Table 1):

Group a: consisted of 121 of the tumerogenic isolates, members of this group were found to be oxidase positive, oxidized lactose to 3-ketolactose, grow on nutrient agar supplemented with 2% MaCl, their reaction to litmus milk was alkaline, acid was produced from sucrose, melezitoes but not from erythritol, propionic acid was reduced to alkali, but not L-tartaric acid; produced pigment on ferric ammonium citrate, variation in sodium citrate utilization in Simmons citrate medium. The reaction of this group to the different tests was identical to the reaction of the reference culture C58 and B6 (*Agrobacterium tumefaciens Biovarl*).

Group b: consisted of 47 isolates, the reaction of these isolates to oxidase varied, did not oxidized lactose to 3-ketolactose did not grow on nutrient agar supplemented with 2% NaCl, their reaction to litmus milk was acidic, acid was produced from erythritol but not from sucrose or melezitose, propionic acid was not reduced to alkali, and did not produce pigment on ferric ammonium citrate medium, the isolates utilized sodium citrate in simmons citrate medium. The reaction of these isolates was identical to the reaction of the reference culture 8302 (*Agrobacterium rhizogense*) (Biovar 2).

Group c: consisted of two isolates, these isolates were found to vary in their reaction to oxidase test and to 3-ketolactose production, they grew on nutrient agar supplemented with 2% NaCl, their reaction to litmus milk was alkali, produced acid from sucrose but not from erythritol or melezitose, they did not reduce propionic acid but reduced L-tartaric acid, they did not produce pigment on ferric ammonium citrate did not replace propionic acid, sodium citrate was utilized in simmons citrate they medium. The reaction of these isolates was identical to the reaction of the reference culture of 5858 (*Agrobacterium vitis*) (Biovar 3).

Group d: thirty isolates were placed into an intermediate group since their reaction to the different tests was found to be unlike the reaction of the above mentioned groups.

Pathogenesity Test:

When a mass of bacterial culture from the obtained isolate was applied to wounded stems of tomatoes and *kalanchoe* seedlings 169 of the tumerogenic isolates induced tumors on tomato seedlings after one month, while 54 of the tested isolates developed tumors after more than one month on *kalanchoe* seedlings (Table 1). Tumors started as swelling in the wounded area increased in size and became fleshy. The developed tumors were similar to the tumors developed on tomato seedlings inoculated with the reference cultures of C58, B6 and J isolate 186. Biovar 1 strains were recovered from all hosts with the exception of pear and grapevine. Nactarine, cherry, apricot, *Cichorium pupumilum*, olive, carob, pomegranate and rose yielded only biovar 1. However, biovar 2 strains originated mainly from peach, plum, almond, apple and pear galls.

In vitro test revealed that 70.5% of *Agrobacterium* strains were sensitive to agrocin 84 Table (2), where the majority of them belonged to biovar 1. While biovar 3 isolates where found to be resistant to agrocin.

Table (1). Characteristics of Jordanian isolates of *Agrobacterium tumefaciens* and pathogenesity tests.

Host	No. of Isolates	Biotype				Pathogenicity tests		Agrocin Sensitivity	
		1	2	3	unbiotyped	Tomato	Kalancho	Resistant	Sensitive
Almond	23	9	5	9	-	23	23	18	5
Apple	5	3	2	-	-	5	-	-	5
Apricot	6	6	-	-	-	5	1	1	5
Carob	7	7	-	-	-	-	-	-	7
Cherry	12	12	-	-	-	12	-	3	9
Cichorium pupumilum	9	9	-	-	-	9	-	-	9
Grapevine	2	-	-	-	2	-	-	2	-
Nectarine	5	5	-	-	-	5	4	-	5
Olive	5	5	-	-	-	5	-	-	5
Peach	80	42	23	15	-	65	20	28	52
pear	4	-	4	-	-	2	2	-	4
Plum	31	12	13	6	-	27	4	7	24
Pomegranate	3	3	-	-	-	3	-	-	3
Rose	8	8	-	-	-	8	-	-	8
Total	**200**	**121**	**47**	**30**	**2**	**169**	**54**	**59**	**141**

Laboratory Sensitivity Test:

All the tested bioagents were found to inhibit the growth of the three tested tumorgenic *Agrobacterium* isolates with various degrees. The largest inhibition zones were formed around the wells filled with garlic extracts, with 50, 47and 56 millimeter diameter in plates already inoculated with J. isolate 186, B6 and C58, respectively, which differed significantly from other tested bioagent treatment, followed by *Bacillus subtilis*, *Penicillium sp*, K84 and *Trichoderma harzianum,* respectively Table (3).

Table(2). Sensitivity of *Agrobacterium* isolates to agrocin.

Agrocin	Biovar 1	Biovar 2	Non-biotyped	Biovar 3
Sensitive	101	27	13	-
Resistant	20	20	17	2
Total	121	47	30	2

The less diameter of inhibition zone was recorded with the suspension of K1026 at 18.7, 15.7 and 17 mm inhibition zones diameter, in plates inoculated with J. isolate 186, B6 and C58 isolates, respectively, which differed significantly from other treatments for the three tested isolates. However, the less susceptible tested strain was found to be B6 and the highest susceptible strain was J. isolate 186 followed by C58 Table (3) where the highest diameters of inhibition were found to be 56.0, 45.7, 32.3 and 32 mm, when the growth of C58 was tested against the extracts of garlic extract, *Pencillium sp, Bacillus subititis* and *Trichoderma harzianum,* respectively.

Table (3). Effect of some bioagents and garlic extracts on growth of some isolates of *A. tumefaciens.*

Bioagents	*Strain*		
	Inhibition zone (mm)		
	J. Isolate 186 *	B6	C58
Bacillus subtilis	34.75 a	26.7 b	32.3 c
Garlic Extract	50.3 a	46.75 a	56.0 a
K1026	18.7 d	15.7 c	17.0 d
K84	26.0 c	25.0 b	30.0 c
Penicillium sp	33.7 b	26.0 b	45.7 b
Trichoderma harzianum	25.75 c	23.7 b	32.0 c
L.S.D.	6.428	6.923	7.050

* Means in the same column followed with the same letter did not differ significantly in Duncan's Multiple Range Test at $p \leq 0.05$.

Preliminary Study on Tomato Seedlings

Dipping the roots of tomato seedlings in the suspension of the different tested bioagents before watering the seedlings with the suspension of the tumorgenic strain resulted in the reduction of tumor formation on the roots. Treating with K1026 was the most effective; where no tumors formed on tomato seedlings inoculated by C58 and Jordanian isolate 186 and the lowest percentage of gall (8.0%) was observed on seedling roots inoculated with B6. However, no tumors were developed on tomato roots dipped into the suspension of K84, garlic extract and *Penicillium sp.*, but 12.5% of the seedlings developed tumors on their roots when their roots were dipped into *B. subtilis* or *Trichoderma harzianum* and their seedlings were watered with the suspension of C85 isolate.

On the other hand, dipping tomato seedling roots into K84, garlic extract and *Penicillium sp.* reduced the percentage of tomato seedling watered with the suspension of B6 and resulted in 15.0, 17.5 and 17.5% galled tomato seedlings, respectively, and those watered with the suspension of Jordanian isolate resulted in 13.5, 15.0 and 20.0%, respectively with no significant differences among them. No significant difference was detected between these bioagents and *Trichoderma harzianum* where the percentages of watered tomato seedlings by the suspension of Jordanian isolate and B6 resulted in 22.5 and 20.0% of the seedlings which showed tumor on their roots, respectively. While the lowest effective bioagent in the reduction of tumor formation when the tomato seedlings were watered with the suspension of *B. subtilis*, where the percentages of infected tomato seedlings watered with B6 and Jordanian isolate suspension resulted in 25.0 and 30.0% infected seedlings, respectively Table (4).

GF677

Dipping the roots of GF677 seedlings in the suspension of the different tested bioagents resulted in a significant reduction of tumor formation on their roots in comparison to control seedlings. However, dipping roots of GF677 seedlings into the suspension of *Trichoderma*, *Penicillium sp.,* and K1026 prevent tumor formation on the roots of GF677 seedlings when the seedlings were watered with the suspension of either C58 or J. isolate 186, which differed significantly from other treatments. *Bacillus subtilis* was found to be less effective and showed 5%, 3.5% and 7% of GF677 seedlings developed tumors on their roots when the seedlings were watered with C58, J. isolate 186 and B6 suspension, respectively.

Table (4). Percentage of infected tomato seedlings when different bioagents used against three isolates of *Agrobacterium tumefaciens.*

Bioagents	***Isolates***		
	J. Isolate 186 *	**B6**	**C58**
Bacillus subtilis	30.0 b	25.0 b	12.5 b
Garlic Extract	15.0 c	17.5 c	0.0 c
K1026	0.0 d	8.0 d	0.0 c
K84	13.5 c	15.0 c	0.0 c
Penicillium sp.	20.0 c	17.5 c	0.0 c
Trichoderma harzianum	22.5 bc	20.0 bc	12.5 b
Control	80.0 a	68.75 a	80.0 a
L.S.D	10.93	6.34	4.08

* Means in the same column followed with the same letter did not differ significantly in Duncan's Multiple Range Test at $p \leq 0.05$.

Table (5). Percentages of Infected GF677 seedlings.

Bioagents	***Strains***		
	J. Isolate *	**B6**	**C58**
Bacillus subtilis	3.5 d	7.0 c	5.0 dc
Garlic Extract	17.5 b	22.0 b	12.5 b
K1026	0.0 d	4.0 c	0.0 d
K84	12.0 c	14.4c	0.0 d
Penicillium	0.0 d	5.0 c	0.0 d
Trichoderma harzianum	0.0 d	3.0 c	0.0 d
Control	100.0 a	58.8a	76.25 a
L.S.D	7.652	5.530	4.583

* Means in the same column followed with the same letter did not differ significantly in Duncan's Multiple Range Test at $p \leq 0.05$.

Garlic extract was found to be less effective in preventing tumor formation on the GF677, and showed 12.5, 17.5 and 22% of those seedlings were watered with the suspension of the C58, Jordanian isolate and B6 were 12.0 and 17.5%, GF677 seedlings showed tumor on their roots when their roots were dipped into K84 and garlic extract, respectively. On the other hand, GF677 seedlings when their roots dipped in the suspension of K84 and garlic extract showed 14.4% and 22% of their seedlings. Their roots developed tumor when they were watered with the suspension of the B6 isolate.

5. DISCUSSION

Crown gall disease caused by the bacterium *Agrobacterium tumefaciens* is one of the important diseases attacking fruit trees in Jordan, the disease is widespread throughout the country and expanded with the expansion of the area planted with fruit trees in the country. Pathogenic *Agrobacterium tumefaciens* isolates were isolated from peach, nectarine, plum, cherry, apricot, almond, apple, pear, *cichorium*, olive, grapevine, carob, pomegranate and roses (Fakori and Khlaif, 1996; Al-Karablieh and Khlaif, 2002).

Biochemical characterization and biotyping of *Agrobacterium* isolates showed that 60% of the isolates belonged to biotype 1, 23.5% belonged to biotype 2, 15% to intermediate biotype, and only 2 isolates were found to belong to biotype 3, and the majority of biotype 1 and 2 were isolated from stone fruits and biotype 3 is restricted to grapes only. Our results are in agreement with the result of (Bouzar *et al*., 1991 and Zonia and Raio (1999) where the majority of biovar 1 isolates were isolated from almond and apricot while the majority of biovar 2 isolates were obtained from peach and biovar 3 isolates were isolated from grapevines only.

Olive, carob, pomegranate and rose isolates were classified as biovar 1, these results are in agreement with the results of Bouzar *et al*. (1991).

Our results showed that a large proportion of *Agrobacterium tumefaciens* isolates (71%) were found to be sensitive to agrocin (84) where the majority of them belonged to biotype 1 and grape isolates of biotype 3 were found to be resistant to agrocin. Because agrocin is sensitive to Ti plasmid-borne, our data suggested that biovar 1strains are most likely to harbor a nopalin (agrocinopine), a type plasmid. Our results in this are in agreement with the results of Bouzar *et al*. (1991).

All the tested bioagents were found to be effective in inhibiting the growth of the three tested strains in plates as well as in reducing the galled tomato and GF677 seedlings. The results on the effect of the different bioagents on the growth of the tested strains were found to follow the same pattern for the same tested strains. Garlic extract was found to be highly effective in reducing the growth of tested strains followed by *Bacillus subtilis*, *Penicillium sp.*, K84 and *Trichoderma harzianum* and the less effective one was K1026.

Variation in the growth inhibition as a result of the tested bioagents showed that the tested strains varied in their susceptibility to the different tested bioagents where the Jordanian isolate 186 and the agrocin C58 sensitive ones were highly susceptible while the B6 isolate agrocin resistant was less susceptible. These results are in agreement with the results of Cooksey *et al*. (1980) where a good correlation between agrocin 84 sensitivity and biological control by K84.

On the other hand, all the tested bioagents reduced the percentage of galled tomato and GF677 seedlings, in comparison to the control. K1026, K84, garlic extract and *Penicillium sp.* prevent tumor formation on the roots of tomato and GF677 seedlings, watered with the suspension of C58 isolate, also the agrocin sensitive C58 and Jordan isolate were the different bioagents which reduced gall formation on seedlings watered with the agrocin sensitive strain C58 and isolate B6 the agrocin resistant strain was less affected by the different bioagents even if the percentage of galled tomato and GF677 seedling was reduced when the seedlings were watered with the B6 strain resistant to agrocin. These results are in agreement with the results of Bouzar *et al*. (1991) and Lopez *et al*. (1987), where K84 protection was found to be effective against agrocin sensitive strain and agrocin resistant strain.

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الخصائص والمكافحة الحيوية لمرض التدرن التاجي في الأردن

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ملخـص

عزلت البكتيريا *Agrobacterium tumefaciens* المسببة لمرض التدرن التاجي من الدراق, اللوز, المشمش, الكرز, النكتارين, الخوخ, التفاح, الكمثرى، الهندباء، العنب, الخروب, الرمان, والورد. ودلت نتائج الاختبارات البيوكيميائية التي أجريت على (200) عزلة من *Agrobacterium* على أن 60% منها تتبع الطراز البيولوجي الأول, و17 % تتبع الطراز البيولوجي الثاني وأن عزلتين عزلتا من العنب تتبعان الطراز البيولوجي الثالث وأن 15% لا تتبع أي طراز بيولوجي. كما وجد أن معظم عزلات الطراز البيولوجي الأول قد عزلت من أشجار اللوزيات بالاضافة الى الزيتون, الخروب, الرمان, الهندباء والورد. كما وجد أن أكثر العزلات انتشارا في الأردن تتبع الطرازين البيولوجيين الأول والثاني.

دلت الدراسة على أن 71% من هذه العزلات كانت حساسة لـ ـ Agrocin ومعظمها تتبع الطراز البيولوجي الأول. كما دلت نتائج الدراسة التي أجريت على تأثير عدد من الكائنات المضادة ومستخلص الثوم، على أن معظمها كانت فعالة في تثبيط نمو العزلات الثلاث المرضية (Jordanian isolate 186, C58 and B6) (الحساسة والمضادة لـ Agrocin) في المختبر, كما كانت فعالة ايضا في خفض نسبة أشتال البندورة وGF677 التي اظهرت أوراما على جذورها بعد تغطيس هذه الجذور في معلقات هذه الكائنات، ومن ثم ريها بمعلق احدى هذه العزلات. كما وجد أن تأثيرها كان فعالا في تثبيط نمو العزلات الحساسة لـ ـ Agrocin، بالإضافة الى العزلة المضادة لتأثير Agrocin.

* قسم وقاية النبات، كلية الزراعة، الجامعة الأردنية. تاريخ استلام البحث 2005/2/15، وتاريخ قبوله 2006/6/8.