

***Bacillus pumilus*, A New Pathogen on Mango Plants**

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Five infective bacterial isolates were isolated from blighted leaves and dieback twigs of mango (*Mangifera indica* L. var. Hindi Bisinnara). All isolates were found pathogenic and were characterized as rod-shaped, Gram positive, endo-spore formers and yellow pigment producers. Otherwise, cellular fatty acids analysis of representative isolate MB₂ confirmed that the obtained isolates are *Bacillus pumilus*. Resulted plants from seeds of inoculated fruits revealed blight leaves and dieback symptoms while, no symptoms were exhibited when mango plants were grown from healthy seeds under soil infestation with the pathogen.

Mango varieties variously responded to infection with *Bacillus pumilus*. Mango var. Hindi Bisinnara was the most susceptible; while Goleck appeared the most resistant mango variety. The tested isolates of *Bacillus pumilus* could infect several hosts range such as leaves of cabbage and peach, and fruits of apricot, apple, cucumber, olive, pepper and squash and flower head of cauliflower, cloves and bulbs of garlic and onion, were infected by the tested isolates.

According to the literature review, this is the first report on the occurrence of *Bacillus pumilus* as a causal agent of leaf blight of mango trees in El-Minia Governorate, Egypt.

Key words: Dieback; leaf blight and *Mangifera indica* L.

Mango (*Mangifera indica* L) is one of the popular seasonal fruits found mainly in the tropical and subtropical countries (Srivastava, 1998). In the last 15 years, there has been a huge increase in mango production as mango markets have been developed in Europe and North America (Lopez and Montesions, 1996).

Unfortunately mango suffers from several diseases at all stages of its life. All the parts of the plant, namely; trunk, branch, twig, leaf, petiole; flower and fruit are attacked by a number of pathogens including fungi, bacteria and algae. They cause several kinds of rot, dieback, anthracnose, scab, necrosis, blotch, spot, mildew (Persley, 1996). The commercial viability of this crop has been threatened by the frequent occurrence of bacterial apical necrosis a newly reported diseases of mango which is caused by the phytopathogenic bacterium *Pseudomonas syringae* pv. *syringae* (Cazorla, 1998 and Cazorla *et al.*, 2002).

Symptoms include necrosis of flower buds, leaves, stems and flower panicles they cause severe economic losses due to the decrease in fruit set. Some bacterial diseases of mango trees caused by *Agrobacterium tumefaciens*, *Racillus subtilis* and

Erwinia carotovora subsp. *carotovora* (Bradbury, 1986) and *Xanthomonas campestris* pv. *mangiferae indica* (the most widely known) and producer of bacterial blotch spot of mango fruits (Bloetz *et al.*, 1994)

In the last few years, symptoms of leaf blight combined with dieback of twigs on mango trees grown in different orchards at western Samalout (a new reclaimed area), Minia Governorate, were observed. Preliminary isolation trials indicated the presence of a bacterial agent associated with the disease. Thus, this work is planned to; 1) isolate and identify the causal agent(s); 2) describe pathological properties and 3) test the varietal response.

Materials and Methods

Isolation of the causal organism(s):

Blighted leaves and dieback twigs of mango (Fig.1A) were collected from different orchards located in western Samalout, a new reclaimed area called Shosha region, Minia Governorate. Diseased samples were individually immersed in 70% alcohol then placed in a sterilized mortar, crushed in 2 ml water with small amount of acid-washed sand then left to stand for 15 min. After that, a loopful from the homogenate of each diseased sample was streaked onto plates contained nutrient agar (NA) medium. Inoculated plates were incubated at 25°C and checked daily for bacterial colony development. Developed single colonies were transferred to slants, kept at 5°C until being used.

Pathogenicity tests:

Pathogenicity trials were performed on mango leaves (var. Hindi Bisinnara). Selected healthy apparent leaves from mango plants grown from disinfested seeds in heat sterilized soils-containing pots (30-cm-diam.) for 3 months. Leaves were subjected to pathogenicity tests using several inoculation methods (e.g. rubbing with carborandum puncturing, wounding and spraying). In case of spray a bacterial suspension (10^8 colony forming unit (CFU)/ml), prepared from 48h old cultures was sprayed directly on leaves until runoff (Goth and Webb, 1981). As for wounding method, wounded leaves were inoculated using a fine entomological needle loaded with bacterial growth of 24h old cultures (Ouf *et al.*, 1987). While rubbing with carborandum was conducted as described by Klement *et al.* (1990). Puncture method was carried out by puncturing the leaves with sterile tooth-picks bearing small portions of 24h old bacterial growth of the tested isolates through these punctures where the tooth-picks were left. Check plants were similarly treated as described for each inoculation methods by using bacterial-free sterile water. Two weeks later, the inoculated leaves were examined for the development of the leaf blight. Reisolation was conducted to confirm Koch's postulates.

Disease assessment:

Blight severity was assayed using an arbitrary 0-5 scale where 0= no symptoms, 1 = 1-25%, 2 = 26-50%, 3 = 51-75 % and 5 = 76%-completely blighted mango leaves. Disease severity index (DSI) was calculated according to the Methods of Vakalounakis (1990) as follows:

$$DSI = \sum d / (d \max \times n) \times 100$$

Whereas: d is the disease rating possible and n is the total number of mango leaves examined in each replicate.

Identification of the pathogens:

Studying their morphological, physiological and biochemical characters identified five bacterial isolates that recommended in the Bergey's Manual (Breed *et al.*, 1974). Also, the determinative scheme of the taxonomy of the genus, Bacillus (Smith *et al.*, 1952). However the tests were carried out as described by Stapp (1961), Dye (1968) and Klement *et al.* (1990).

Fatty acid analysis:

A representative isolate (MB₂) was sent to Dr. J. Janse, Plant Protection Service, Wageningen, Netherlands, for fatty acid analysis to confirm the identification. The computerized final data of percentages of different fatty acids using model 101 system, and also, homology percentage of fatty acid analysis of the concern isolate comparing with different reference isolates were received.

Seed inoculation and soil infestation:

Healthy apparent fruits of mango var. Hindi Bisinnara were surface desinfected by immersing them in 1% sodium hypochlorite for 3 min. Then washed thoroughly 3 times by sterilized distilled water and separately with 1 ml of bacterial suspension that prepared as mentioned in pathogenicity test. Fifteen days later, seed of artificially inoculated fruits or uninoculated ones (control) were sown in pots 30-cm-diam. containing sterilized soil (4 seeds/pot) and five pots were used for each isolate. The experimental work was done in a greenhouse at approximately 30-35°C during the day.

As for soil infestation, steam sterilized soil pots were infested with bacterial suspension (to obtain 10⁶ CFU/ g soil) which prepared as described above. Surface sterilized seeds of mango were sown two days later. After 3 months of planting, germinated seeds were counted (Strandberg and White, 1989) and the resulted plants were evaluated for developing any symptoms on damping off, blighting leaves or dieback.

Response of mango varieties to infection:

Leaf blades of mango plants which sown under the same condition of the above experiment (similar to the control of soil infestation) were inoculated with bacterial suspension using carborandum rubbing methods (Klement *et al.*, 1990). Check plants were treated similarly with bacterial-free solution of 0.1% methylcellulose. Inoculated plants were covered with plastic bags for 48 h at 30-35°C in the greenhouse and daily examined. Disease severity was calculated 15 days after inoculation according to the Method of Vakalounakis (1990), as described above.

Host range:

Organs from various plants (Table 7) were inoculated with each of the five isolates to determine their host range. Storage roots, fruits, tubers, bulbs and cloves of the tested plants were inoculated by injection of 200µl bacterial suspension per plant organ. Inoculated organs were kept in sterile containers, each supplemented

with a sterile moist cotton pad at room temperature (about 30°C). In case of leaf inoculation, the inoculated plants were kept at 30°C in the greenhouse where 5 samples or plants were used. Control samples or plants were similarly tested with sterile water only and kept at the same conditions.

Statistical analysis:

Data were transformed from percentages to arcsines and then subjected to statistical analysis to determine the standard deviation (SD) and least significant differences (LSD) were calculated to compare variances between treatments (Gomez and Gomez, 1984).

Results and Discussion

Pathogenicity test:

Five infective bacterial isolates were isolated from blighted mango leaves and dieback mango twigs (Table 1 and Fig.1B). Isolates appeared various virulence where depended on inoculation methods and isolates tested isolate MB₂ gave the highest virulence effect (65% blight severity) under puncture inoculation methods and isolate MB₁ showed the weakest virulence effects (25% blight severity). The rest of bacterial isolates reacted as intermediate virulent ones. Under spray inoculation with all isolates were failed to incite symptoms indicating that the obtained isolated are need wound to infect mango leaves data are consistent with those reported by Gabr and Gazar (1983) and Saleh *et al.* (1997).

Table 1. Blight severity to mango var. Hindi Bisinnara leaves induced by various bacterial isolates as influenced by different inoculation methods

Isolate No.	Source of isolate	Blight severity (%) incited by;			
		Rubbing	Wounding	Puncturing	spraying
MB ₁	Mango leaf	30 ± 2.0	25±4.0	20±1.2	0.0
MB ₂	Mango leaf	65 ± 4.0	55±3.0	45±2.5	0.0
MB ₃	Mango twig	50 ± 3.0	45±5.0	30±1.8	0.0
MB ₄	Mango leaf	55 ± 2.5	40±3.0	30±2.0	0.0
MB ₅	Mango twig	40 ± 3.0	40±4.0	25±1.5	0.0

Identification of the pathogens:

The morphological, physiological and biochemical characters of the obtained bacterial isolates, exhibited similar properties (Table 2). They were rod-shaped, measurements. Gram positive, endo-spore formers and yellow pigment producers. The positive tests were: catalase, methyl red reaction, aerobiosis, H₂S production and hypersensitive reaction on tobacco leaves. All isolates were utilized galactose, glucose, inositol, mannitol, trehalose and xylose while weakly utilize glycerol and reacted negative for aesculen hydrolysis, starch hydrolysis, nitrate reduction, V.P. test, indole formation, levan production and gelatin liquefaction. Also, all isolates

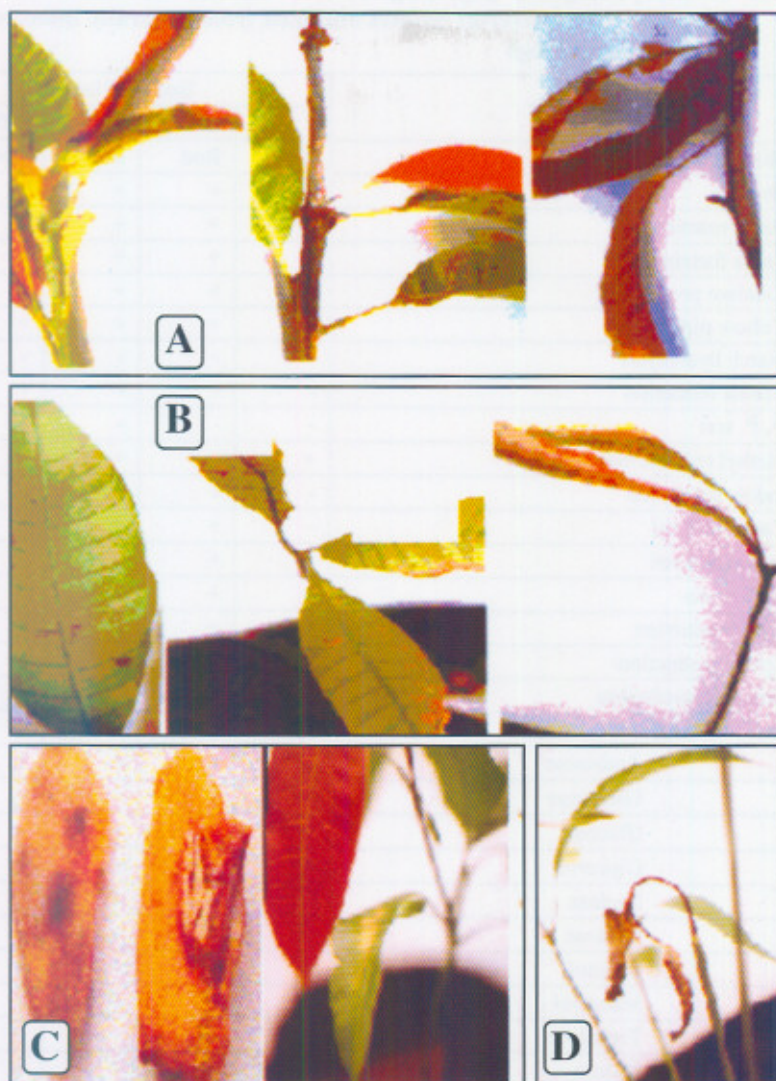


Fig. 1. A) From left to right naturally blighted leaves , die backed twig and blighted leaves combined with die backed twig of mango (*Mangifera indica* var. Hindi Bisinnara); B) Artificially inoculated leaves and twigs of mango plants by *Bacillus pumilus* isolate MB₂ showing water soaked lesion (left) developed to blight (medium) and dieback twig (right) and C) Seed of artificially inoculated mango fruits by *B. pumilus* isolate MB₂ (left), note that these seeds when they had been germinated resulted in blighted leaves (medium) and die backed twig (D).

Table 2. Morphological, physiological and biochemical characters of the pathogenic bacterial isolates obtained from naturally infected mango leaves and twigs

Character	Bacterial isolate				
	MB ₁	MB ₂	MB ₃	MB ₄	MB ₅
Shape of cell	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+
Gram reaction	+	+	+	+	+
Spore forming	+	+	+	+	+
Catalase production	+	+	+	+	+
Yellow pigment	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-
Nitrate reduction	-	-	-	-	-
V. P. test	-	-	-	-	-
Methyl red reaction	+	+	+	+	+
Indole formation	-	-	-	-	-
Carrot soft rot	-	+	+	-	+
Potato soft rot	-	+	+	-	+
Aerobiosis	+	+	+	+	+
H ₂ S production	±	±	+	±	±
Levan production	-	-	-	-	-
Gelatin liquefaction	-	-	-	-	-
Utilization of,					
Arabinose	-	-	±	-	-
Galactose	+	+	+	+	+
Glucose	+	+	+	+	+
Glycerol	±	±	±	±	±
Lactose	-	-	-	-	-
Maltose	-	-	-	-	-
Inositol	+	+	+	+	+
Mannitol	+	+	+	+	+
Trehalose	+	+	+	+	+
Xylose	+	+	+	+	+
Hypersensitive reaction on tobacco leaves	+	+	+	+	+
Sensitivity to antibiotics,					
Erythromycin 50 µg	R	R	R	R	R
Penicillin 50 µg	S	S	S	S	S
Streptomycin 50 µg	S	S	S	S	S
Aesculen hydrolysis	-	-	-	-	-

(+)= Positive reaction; (-)= Negative reaction; (±)= Weekly reaction; (R)= resistant and (S)= Sensitive.

did not utilize arabinose, lactose and maltose. All are resistant to 50µg erythromycin but sensitive to 50 µg penicillin and streptomycin. Identification trials revealed that the five bacterial isolates reacted similarly with the majority of the tested characters and suggested that they all are *Bacillus pumilus* as reported previously (Wolf and Barker, 1968; Schaad, 1980 and Gabr and Gazar, 1983).

Fatty acid analysis:

To confirm the identification, one represented isolate MB₂ was subjected for fatty acid analysis. Results showed that cellular of the bacterium *Bacillus pumilus* isolate MB₂ contained 12 different fatty acids (Table 3). The isolate is rich with saturated fatty acids of 15 carbon atoms, they contained 61.15 % from 15 carbon atom ISO saturated fatty acids, followed by 15 carbon ANTE: ISO saturated 16.95%.

Table 3. Analysis of cellular fatty acids of *Bacillus pumilus* isolate MB₂

Fatty acid *	Cellular fatty acids (%)
13: 0 ISO	0.92
15: 0 ISO	1.62
14: 0	0.87
15: 0 ISO	61.15
15: 0 ANTE: ISO	16.95
16: 0 ISO	2.47
16: 1 W7C alcohol	0.90
16: 0	2.30
17: 1 ISO W/C	2.39
17: 0 ISO	6.04
17: 0 ANTE ISO	2.56
SU UME D Feature	0.47

* Number of carbon atoms: O (saturated) or unsaturated fatty acid.

Seed inoculation and Soil infestation:

Data in Table (4) show significant differences between seed inoculation and soil infestation by all bacterial isolates tested. Seed inoculation revealed damped-off mango plants var. Hindi and isolate MB₂ was the most aggressive. Resulted plants from seeds of singly inoculated fruit (Fig. 1C) by isolate MB₂ of *B. pumilus* revealed the highest average of blight leaves and dieback symptoms (Fig. 1D) to mango plants as compared to other isolates. By seed inoculation, isolate MB₂ caused 25% damping-off, and 40% leaf blight severity while isolate MB₃ was the least aggressive since it induced 5% damping-off, 20% leaf blight severity. As for soil infestation neither damping off nor blight disease were revealed. Data indicate that this bacterium may be disseminated through seeds but not by soil infestation.

Table 4. Mean percentage of damping off and blight severity to leaves of mango plants var. Hindi

Bacterial isolate	Seed inoculation		Soil infestation	
	Damping- off	Leaf blight	Damping- off	Leaf blight
MB ₁	10 ± 0.5	20 ± 1.2	0.0	0.0
MB ₂	25 ± 1.5	40 ± 1.8	0.0	0.0
MB ₃	15 ± 0.6	35 ± 2.1	0.0	0.0
MB ₄	10 ± 0.4	30 ± 1.5	0.0	0.0
MB ₅	5 ± 0.4	20 ± 1.2	0.0	0.0

Data are means of 3 replicates (10 seeds per each) ± SD

Reaction of mango varieties to Bacillus pumilus:

Significant variances were obtained between mango varieties in response to infection with different isolates of *B. pumilus* (Table 5). Mango var. Hindi Bisinnara was the most susceptible to *B. pumilus* infection (40% blight severity) followed by var. Balady (32%), Alphonse (31%) and Mabrouka that showed average of (30%) blight severity. A moderate reaction to *B. pumilus* infection was recorded when leaves of Mango Timour (24% blight severity) were inoculated. Mango var. Goleck showed a sufficient resistance against *Bacillus pumilus* infection since 18% blight severity was exhibited followed by mango var. Dabsha (14% blight severity) and Khad El-Gameal and Zebdia (12% blight severity), MB₂ was the most significant virulent while gave average 35.5% blight severity and exhibited the highest blight severity (60%) toward mango leaves of Hindi Bisinnara variety. Isolates MB₃ and MB₄ showed moderate infection (24 and 23.5% blight severity, respectively) followed by MB₁ isolate (showed average 21% blight severity). Otherwise, isolate MB₅ provided the least virulent (incited average of 17.5% blight severity) and formed 5% blight severity to leaves of mango var. Goleck.

Bacillus species are common in soil and some may be involved in post-harvest decay of plant tissues *B. pumilus*, *Pseudomonas fluorescens*, *Corynebacterium*, *Erwinia carotovora* subsp. *atroseptica* and other *bacillus* spp. are known to cause bacterial soft rot to storage crops (Ciampi *et al.*, 1976 and Ciampi and Huguélet, 1979)

Bacillus pumilus, *B. subtilis* and *B. polymyxa* are reported to cause post-harvest soft rot of vegetables (Chiu *et al.*, 1964). Gaber and Gazar (1983) reported that these bacteria (*B. pumilus* and *B. polymyxa*) were causal pathogens of head rot of cabbage. *B. pumilus*, *B. subtilis*, *B. coagulans* and *B. polymyxa* have recently been reported to be the main causal agent of garlic cloves post-harvest decay (Saleh, 1995 and Galal *et al.*, 2002). *B. pumilus* causes brown spots on fruit and leaves of Balady peach (Saleh *et al.*, 1997)

Table 5. Blight severity (%) to leaves of various mango varieties caused by inoculation with different isolates of *Bacillus pumilus*

Mango varieties	Blight severity (%) caused by bacterial isolates					Mean
	MB ₁	MB ₂	MB ₃	MB ₄	MB ₅	
Alphonse	25	40	35	30	25	31
Balady	35	40	30	30	25	32
Dabsha	10	20	15	15	10	14
Ewais	20	40	35	35	20	30
Galeck	5	15	10	5	5	8
Hindi Bisinnara	30	60	40	35	35	40
Khad El-Gameal	15	25	15	20	10	17
Mabrouka	30	25	25	30	15	30
Timour	20	35	25	20	20	24
Zebdia	20	30	10	15	10	17
Mean	21	35.5	24	23.5	17.5	--
LSD at 0.05 for: isolates (A)= 5.4; Varieties (B)= 7.2 and Interaction A x B= N.S.						

Host range:

Under artificial inoculation, data (Table 6) reveal that all isolates caused leaf blight of cabbage and peach as well as fruit rot of apricot, apple, cucumber, olive, pepper and squash. Also, head of cauliflower and cloves as well as bulbs of garlic and onion, respectively, were infected by the tested isolates, while potato tubers and legume pods (bean, faba bean, lupin, and soybean) and almond fruit were not affected. Leaves of apple, almond, pear, tomato, pepper, olive, bean, faba bean, cucumber were not infected. Data indicated that *B. pumilus* has wide range under artificial inoculation especially to fleshy tissue. Data are not completely consistent with those reported by (Gabr and Gazar, 1983 and Saleh *et al.*, 1997). However, on the base of previous studies (Gabr and Gazar, 1983 and Saleh *et al.*, 1997) host range experiment declared that *B. pumilus* may has different strains that may cause difficult control such kind of these phytopathogenic bacteria. In addition, intercropping otherwise plant species with mango plants may lead to increase the opportunities of infection with such disease.

Table 6. Host range of *Bacillus pumilus* isolated from blighted leaves and dieback twigs of mango plants

Host	Inoculated organ	Isolate				
		MB ₁	MB ₂	MB ₃	MB ₄	MB ₅
Apricot (<i>Prunus armeniaca</i>) cv. Balady	Fruit	+	+	+	+	+
	Leaf	+	+	+	+	+
Almond (<i>Prunus amygdalus</i>) cv. Balady	Fruit	-	-	-	-	-
	Leaf	-	-	-	-	-
Apple (<i>Malus domestica</i>) cv. Anna	Fruit	+	+	+	+	+
	Leaf	-	-	-	-	-
Bean (<i>Phaseolus vulgaris</i>) cv. Giza 6	Leaf	-	-	-	-	-
	pod	-	-	-	-	-
Cabbage (<i>Brassica oleracea</i>) cv. Balady	Leaf	+	+	+	+	+
Carrot (<i>Daucus carota</i>) cv. Balady	Storage root	-	-	-	-	-
Cauliflower (<i>Brassica oleracea</i> var. <i>botrytis</i>) cv. Balady	Head	+	+	+	+	+
Cowpea (<i>Vigna unguiculata</i>) cv. Cream 7	Leaf	-	-	-	-	-
	Pod	-	-	-	-	-
Cucumber (<i>Cucumis sativus</i>) cv. Medina	Fruit	+	+	+	+	+
	Leaf	-	-	-	-	-
Faba bean (<i>Vicia faba</i>) cv. Balady	Leaf	-	-	-	-	-
	Pod	-	-	-	-	-
Garlic (<i>Allium vineale</i>) cv. Balady	Bulb	+	+	+	+	+
Lupin (<i>Lupinus termis</i>)	Leaf	-	-	-	-	-
	Pod	-	-	-	-	-
Okra (<i>Hibiscus esculentus</i>) cv. Balady	Leaf	-	-	-	-	-
	Pod	+	+	+	+	+
Olive (<i>Olea europea</i>) cv. Balady	Fruit	+	+	+	+	+
Peach (<i>Prunus persica</i>) cv. Balady	Fruit	+	+	+	+	+
	Leaf	+	+	+	+	+
Pear (<i>Pyrus communis</i>) cv. Leconte	Fruit	-	-	-	-	-
	Leaf	-	-	-	-	-
Pepper (<i>Capsicum annum</i>) cv. California wonder	Fruit	+	+	+	+	+
	Leaf	-	-	-	-	-
Potato (<i>Solanum tuberosum</i>) cv. Diamant	Tuber	-	-	-	-	-
Soybean (<i>Glycine max</i>) cv. Balady	Leaf	-	-	-	-	-
	Pod	-	-	-	-	-
Squash (<i>Cucurbita</i> sp.) cv. Escandranij	Fruit	+	+	+	+	+
Sugar beet (<i>Beta vulgaris</i>) cv. Oskarpoly	Root	+	+	+	+	+
Sweet potato (<i>Ipomea batata</i>) cv. Balady	Root	-	-	-	-	-
Tomato (<i>Lycopersicon esculentum</i>) cv. Super Marmand	fruit	-	-	-	-	-

(+)= Positive infection and (-)= Negative infection

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البكتريا باسيلس بوميلس (*Bacillus pumilus*)

مسبب مرضى جديد لنباتات المانجو

نور عبد العزيز جلال* وعلى عبد المنعم البنا* ولباب يقمى**

* قسم امراض النبات - كلية الزراعة - جامعة المنيا - المنيا - مصر.

** قسم البكتريولوجى - معهد وقاية النبات - واجنجن - هولندا.

تم عزل خمس عزلات بكتيرية ممرضة من أوراق وأفرع غصنه مصابة باللحفة وبالموت الرجعى لنبات مانجو هندي بسنارة على التوالي. كل العزلات كانت ممرضة جرحيا وتميزت العزلات بانها عصوية الشكل موجبة لصبغة جرام ومتجرثمة وتنتج صبغة ذات لون أصفر بالإضافة الى ذلك فإن تحليل الأحماض الدهنيه لخلايا العزلة البكتيرية MB₂ أكدت ان العزلات المتحصل عليها هي تابعة للبكتريا باسيلس بوميلس أظهرت النباتات النامية من بذور (مأخوذة من ثمار معدية صناعيا بالبكتريا) اعراض لفحة اوراق وموت رجعى للبادرات فى حين انه لم تظهر أى أعراض إصابة على النباتات النامية من بذور سليمة تم زراعتها فى تربة معدية بالبكتريا

تبانت إستجابة أصناف المانجو للعدوى بالبكتريا باسيلس بوميلس حيث أظهر الصنف هندي بسنارة اعلى قابلية للأصابة فى حين أظهر الصنف جاليك اعلى مقاومة. وجد أن للبكتريا المختبرة لها مجال عوائلى واسع حيث أنها أحدثت إصابة على أوراق نباتات الكرنب والخوخ وعفن لثمار المشمش والتفاح والخيار والزيتون والفلفل والكوسمة والقرص الزهري للفتيظ والبصل وقصوص الثوم.

ووفقا للمراجع المتاحة فإن هذه الدراسة تعتبر أول تسجيل للبكتريا باسيلس بوميلس كمسبب مرضى على نباتات المانجو فى محافظة المنيا - مصر.