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STATUS OF FIRE BLIGHT (*Erwinia amylovora* Burr. Wilson *et al.*) IN Egypt: DISTRIBUTION, IDENTIFICATION AND CONTROL

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ABSTRACT

Fire blight has been a serious problem and destructive disease in pear orchards. The first appearance of the disease at Mammal El-Kezaz in the region between Alexandria and Damanhur provinces was proved in 1964. However, in 1982 sever outbreak of fire blight in the Nile Delta of Egypt was noticed. The disease spread rapidly in most governorates and as a consequence of the disease numerous orchards has been eradicated.

The identification of twenty-three isolates obtained during this study was preformed by traditional bacteriological tests, BIOLOG system and serological techniques (dotimmunobinding assay, DIA and immunoflourescens test, IF). Diseased pear tissues with typical fire blight symptoms showed positive reaction with DIA and IF using the specific antiserum of *Erwinia amylovora*.

All tested chemical compounds and antibiotics decreased growth of *E. Amylovora* and disease severity on immature pear fruitlets, *in vitro*. Starner 20% (oxolinic acid) and nalidixic acid 20% were the most effective on the growth of *E. Amylovora* and disease severity. Meanwhile, Kocide 101, Kocide 2000, Copper oxychloride 50%, Agrimycin 17, (Strptomycin sulfate), Apromycin 20% were moderately effective and Kasugamycin was less effective.

Field experiments were conducted using Le Conte pear cultivar during 2001, 2002 growing season at Kalubia governorate. Integration between organic manure, mineral fertilizers (NPK) and Agrimycin 17 or Starner 20% greatly reduced the number of infected clusters and significantly increased pear yield compared with the control treatment. No differences were found between the copper compounds which used spraying in dormancy stage. In order to develop a management strategy to control fire blight in orchards, an integrated approach was adopted using organic manure, mineral fertilizers and bactericides during blooming.

INTRODUCTION

Fire blight caused by *Erwinia amylovora (Burrill) Winslow et al.* is considered the most destructive disease of pear. The initial discovery near Mammal El-Kezaz in the region between Alexandria and Damanhur El-Helaly *et al.* (1964) and the severe outbreak of the disease in the Nile Delta at Kumbeneit Abo-Keer, Behera governorate in 1982, (Abo El-Dahab *et al.* 1983) and since then, the disease spread rapidly through different provinces and was responsible for serious economic losses to pear growers.

The diagnostic and identification tests were preformed on all isolates from different provinces by using Biolog System (Tawfik *et al.* 2000). Several serological techniques, useful for detection or identification

of *E. Amylovora* in infected plants or pure cultures were assayed using selected antibodies against the whole cells of *E. Amlovora*. The ability of the antibodies to react in different serological techniques allows a specific and sensitive detection of *E. Amylovora* (Garris *et al.*, 1996).

Recently and at present, most fire blight control efforts have been directed towards preventing successful establishment of *E. amylovora* in host tissue by spraying with antibiotics or copper compounds (Goodman, 1954; Zeller, 1980; Zwet and Mikhail 1984; Spitko and Alvarado, 1999; Shtienberg *et al.* 2001 and Tawfik *et al.* 2002), these bactericides are usually sprayed when environmental conditions are favorable for the development of fire blight infection. In addition removal of blighted branches during dormancy, less nitrogen fertilizers and management of soil moisture were used to minimize the dissemination of the bacteria through the tree and considered as one integrated measure (Miller and Schroth, 1972; Zwet and Beer, 1995 and Koseglu *et al.* 1996).

The present study aimed to investigate the identification of isolates from different localities by using Biolog system and serological techniques. Also the effect of organic and mineral fertilizers as well, bactericides in suppression the disease.

MATERIALS AND METHODS

Isolation of the bacterium:

Isolation was carried out from blighted flowers, leaves and branches on plates of medium MS according to Miller and Schroth (1972). Single colonies were purified by repeated streaking on NGA.

Pathogenicity tests:

The pathogenic ability of twenty-three isolates from different localities was tested on green immature pear fruit according to the procedures outlined in Schaad (1988). Pathogenicity assay were conducted twice with all isolates.

Characterization of the bacterium:

Gram stain, physiological and biochemical characterization were preformed using Biolog Bacterial Identification System (Jones *et al.*, 1993). Preparation of antigen and antiserum according to Carpenter (1965) from isolate EAK7 which identified by Biolog System for serological assays. Dot-immunobinding assay (DIA) was adopted by Jahn *et al.* (1984) carried out to identify the isolated bacterium by clearly defined colour in the spot using fast violet stain. Indirect immunofluorescens assay (IFA) was carried out according to Anon (1987).

Disease control:

Testing the effect of seven chemical compounds and four antibiotics on the growth of *E. amylovora in vitro* were evaluated by the use of filter paper disc method. Chemical compounds and antibiotics used *in vitro* shown in Table (1).

Table (1): Chemical compounds used in experiment to control fire blight in the field under natural infection.

Common name	Commercial name	Active ingredient %	Dosage
Copper oxychloride	Cobax	50	500 g/100 L
Benalaxyl+Copper oxychloride	Galben copper 46%	11+35	250 g/100 L
Dimesomorph+Copper oxychloride	Acrobat copper 46%	11+35	200 g/100 L
Metalaxyl+Copper oxychloride	Ridomel plus 50%	15+35	250 g/100 L
Streptomycin sulfate	Agrimycin 17	21.2	120 ppm
Kasugamycin+Vethaled	Kasumin	1.2+15	65 ml/100 L
Oxolinic acid	Starner	20	150 g/100 L

Field trials for evaluating the effect of copper compounds and antibiotics for disease control were performed in orchard located at Toukh, Kalubia governorate during 2001 and 2002 seasons on 20 years old Le Conte pear cultivar grafted on *Pyrus communes*. Trees of uniform size were chosen at random (in complete randomized split-split plot design with ten replicates for each treatment).

Farm-yard manure and calcium superphosphate 15% P_2O_5, were used at rates of 75 and 1.5 kg/tree respectively. Ammonium nitrate (33.3% nitrogen) and potassium sulfate (48% K_2O) were applied at the rate of 1 and 0.5 kg/tree, and NPK at the same rates was also applied. Bactericides namely Agrimycin (streptomycin sulfate 21.2%) at the rate of 0.5g/lit, kocide 101 (copper hydroxide 50%) at the rate 1.5g/lit and Starner (oxolinic acid 20%) at the rate 1.5 g/lit were sprayed on blooming.

Estimating the degree of disease severity was recorded at the end of May as disease incidence (Number of infected clusters per tree) according to El-Zayate *et al.* (1995).

RESULTS

Isolation and pathogenicity:

The casual organism was isolated from bacterial exudates (ooze) coming out on the petioles of leaves and blossoms, which collected from Behara, Ismaelia, Kalubia and Menufia governorates in the spring of 1999-2002 seasons. Twenty-three isolates produced on MS medium, reddish, orange colored colonies with deep orange centers, which were indicative of *Erwinia amylovora*.

All the isolates were examined for their pathogenicity on immature pear fruitlets, the results were demonstrated that all isolated were pathogenic with no substantial differences in degree of oozing Table (2). Different isolates showed blackened areas with drops of oozes on pear fruitlets Fig (1).

Table (2): Pathogenicity test for isolated bacterium from pear samples with a typical fire blight symptoms, during 1999-2002 growing seasons, from different governorates in Egypt.

Governorate	Isolate number	Locality	Season	Pathogenicity
El-Behera	EAB1	Kafr El-Dawar	1999	+
	EAB2	Kafr El-Dawar	2002	+
	EAB3	Kafr El-Dawar	2002	+
	EAB4	Kafr El-Dawar	2002	+
	EAN1	El-Noubaria	2001	+
El-Ismailia	EAS2	El-Kasacien	1999	+
	EAS3	El-Kasacien	2000	+
	EAS4	El-Kasacien	2000	+
	EAS5	Gamiet El-Asher	2002	+
	EAS6	Gamiet El-Asher	2002	+
El-Kalubia	EAK6	Toukh	1999	+
	EAK7	Toukh	2001	+
	EAK9	Toukh	2001	+
	EAK10	El-Kanater El-Khairiah	2002	
	EAK11	El-Kanater El-Khairiah	2002	
El-Menufia	EAM1	Ashmoon	1999	+
	EAM2	Ashmoon	1999	+
	EAM4	Ashmoon	2000	+
	EAM6	Ashmoon	2000	+
	EAM7	Ashmoon	2001	+
	EAM8	Ashmoon	2001	+
	EAM9	Ashmoon	2002	+
	EAM10	Ashmoon	2002	+

\+ = Oozing in immature pear fruits.
\- = No oozing in mature pear fruits.

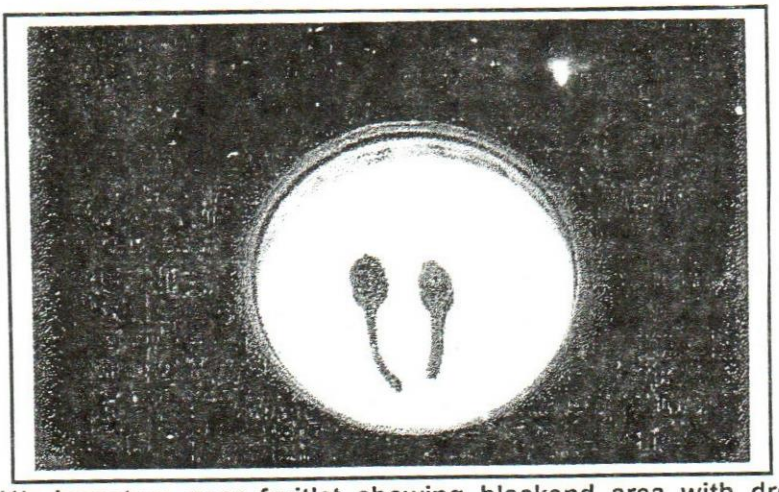

Fig (1): Immature pear fruitlet showing blackend area with drop of bacterial ooze after inoculated with *Erwinia amylovora* (E) and immature pear fruitlet inoculated by sterile distilled water as control (C).

Biolog Microplate System:

Two isolates namely EAK7 and EAM6 were selected from the isolated bacteria, the metabolic capabilities to utilize 95 different carbon sources were examined by the Biolog System. Results showed that *E. amylovora* isolates utilize D- glucose, D- fructose, D-mannitol, trhalose, glucose-1-phosphate, sucrose, D- sorbitol and glucose-6-phosphate, the two isolates were identified as *E. amylovora* according to the computer sheet.

Serological tests:

Antiserum of *E. amylovora* was applied using dot-immunobinding assay and immunofluorscent reaction Table (3) and Figs. (2 and 3). All the isolates of *E. amylovora* and diseased pear tissues gave positive reaction with DIA (clear violet color with high reaction at 1/10 samples dilution). Immunofluorescent test where the bacterial cells which stained with specific fluorescein conjugated antiserum were observed bright green halo around the cell wall using an ultraviolet light source. Meanwhile, healthy pear tissues gave negative reaction using the two tests.

Table (3): Application of *Erwinia amylovora* antiserum on different samples, using dot-immunobinding assay and immuno-fluorescent reaction.

Sample	Source	Dot-immunobinding assay Sample dilution			Immuno-fluoresent test
		1/1000	1/100	1/10	
Isolates of *E. amylovora*:					
EAK7	Pure culture	+	++	+++	F
EAR3	Pure culture	+	++	+++	F
EAB1	Pure culture	+	++	+++	F
EAK9	Pure culture	+	++	+++	F
EAM2	Pure culture	+	++	+++	F
EAM6	Pure culture	+	++	+++	F
EAN1	Pure culture	+	++	+++	F
EAS2	Pure culture	+	++	+++	F
EAS3	Pure culture	+	++	+++	F
Isolates of different species of bacteria:					
Pseudomonas syringae pv. *syringae*	Pure culture	-	-	±	O
Erwinia herbicola	Pure culture	±	+	+	O
Bacillus subtilis	Pure culture	-	±	+	O
Agrobacterium tumefaciens	Pure culture	±	±	±	O
Pear tissues:					
HT	Healthy pear tissues				O
DT1	Diseased pear tissues*	+	++	+++	F
DT2	Diseased pear tissues*	+	++	+++	F
DT3	Diseased pear tissues*	+	++	+++	F
Control	Distilled water				O

* With a typical fire blight symptoms.
- = Negative reaction, ± = Slight reaction, + = Moderate reaction,
++ = High reaction, +++ = Very high reaction.
F = Fluorescence (bright fluorescing rod).
O = No fluorescence.

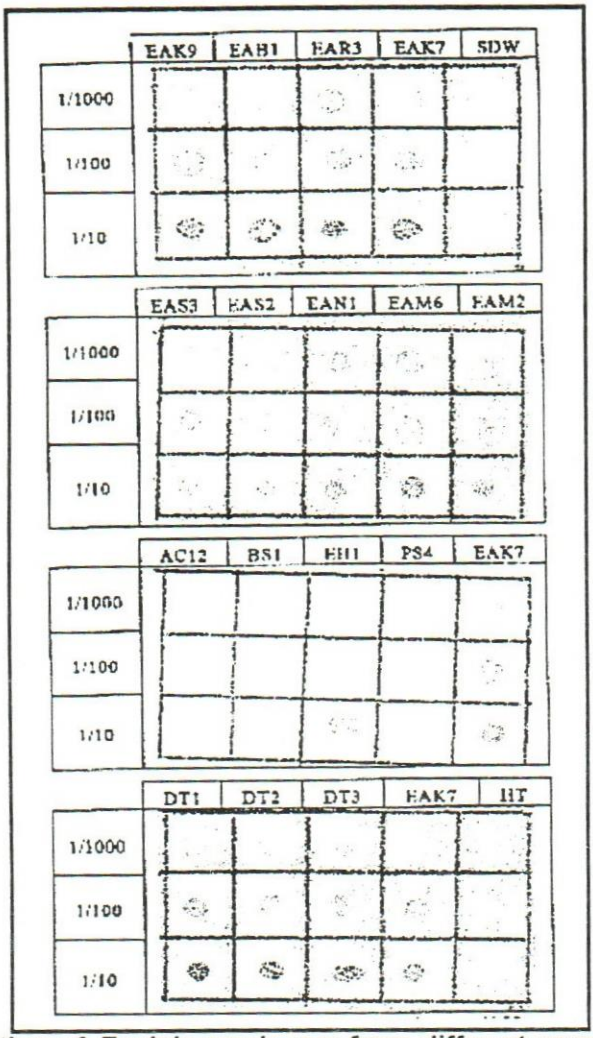

Fig (2): Detection of *Erwinia amylovora* from different samples, using dot-immunobinding assay on nitrocellulose membrane.

EA = Isolates of *Erwinia amylovora.*
SDW = Sterile distilled water.
AC12 = *Agrobacterium tumefaceins.*
BSI = *Bacillus subtilis.*
EH1 = *Erwinia herbicola.*
PS4 = *Pseudomonas syringae* pv. *syringae.*
DT = Diseased pear tissues.
HT = Healthy pear tissues.
1/1000, 1/100, 1/10 = Samples dilution.

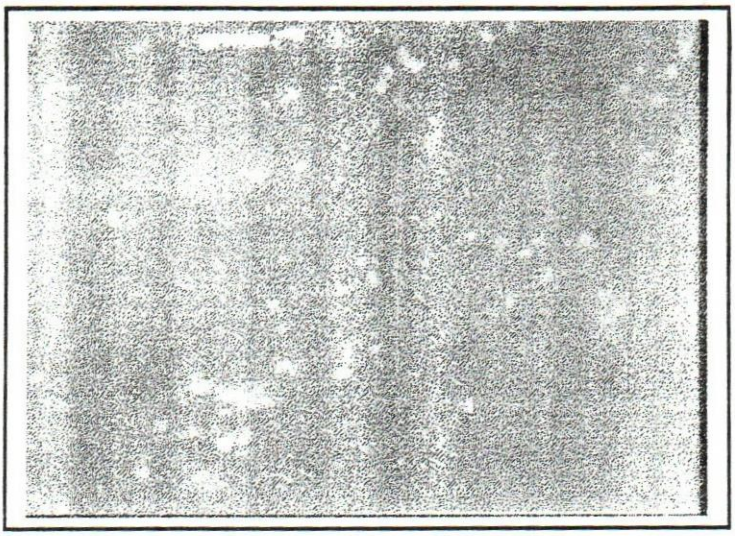

Fig (3): Photomicrograph of bacterial cells of *Erwinia amylovora* stained with specific fluorescein-labelled antiserum; fluorescence is stimulated by ultraviolet light from the mercury lamp. The apple-green fluorescent cell walls were observed.

Disease control:

a. Effect of chemical compounds and antibiotics on growth of *E. amylovora in vitro*:

Results in Fig (4) showed that both chemical compounds and antibiotics decreased the growth of *E. amylovora* compared with the control. Starner 20% and nalidixic acid 20% were the most effective to reduce the growth of the bacterium. Meanwhile, Mycosheilde Agrimycin 17, Kocid 101 and copper oxychloride 50% were moderately effective. However, Kasumin was less effective.

b. Orchard experiments:

1. Integration between organic and mineral fertilizers:

Data in Table (4) showed that all treatments reduced severity of fire blight and increased the productivity compared with the control. The most effective treatment was detected in the interaction between organic manure and NPK. However, application of organic manure or mineral fertilizers alone was less effective.

2. Integration between bactericides and different fertilizers:

Data in Table (5) noticed that the interaction between Agrimycin 17 and Starner 20% and NPK were more effective than interaction Kocid 101 and NPK.

3. Interaction between organic manure, mineral fertilizers and bactericides:

Interaction between organic manure, NPK and Agrimycin 17 or Kocide 101 or Starner 20% greatly reduced severity of fire blight disease and significantly increased pear yield compared with the control in season 2001 and the same trend in season 2002 (Table, 6).

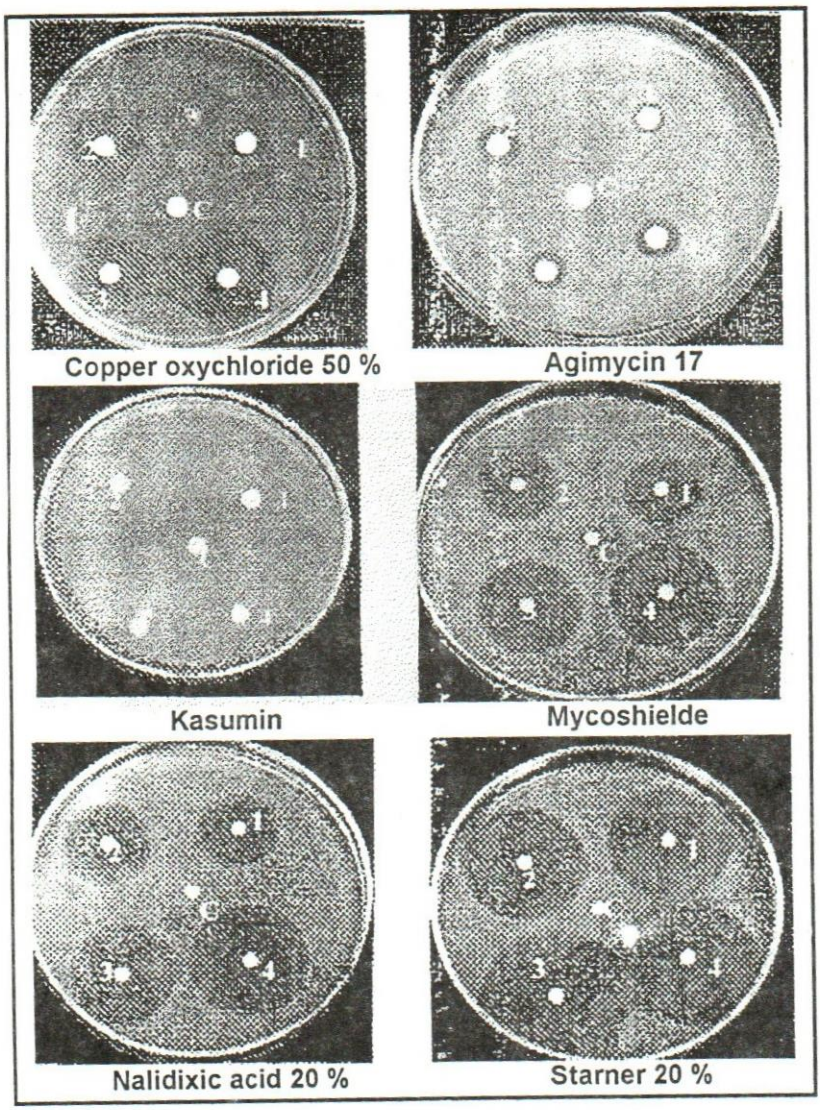

Fig (4): 1, 2, 3 and 4 = doses from low to high.
C = control (sterile distilled water).

Table (4): Effect of organic manure and mineral fertilizers on severity of fire blight and fruit production of pear during 2001 and 2002 growing seasons, at Toukh (Kalubia) region, under natural infection conditions.

Organic manure [1]	Mineral fertilizer [2]	Season 2001			Season 2002		
		Infection[3]	Disease Reduction (%)	Yield[4]	Infection[3]	Disease Reduction (%)	Yield[4]
Treated	Untreated	42.7	4.9	16.3	41.6	5.7	17.2
	Ammonium nitrate (N)	31.3	30.3	27.4	30.0	32.0	28.9
	Calcium superphosphate (P)	28.7	36.1	29.0	28.0	36.5	30.0
	Potassium sulfate (K)	29.4	34.5	28.7	28.7	34.9	29.4
	NPK	21.4	52.4	34.8	21.0	52.4	35.9
Untreated	Ammonium nitrate (N)	41.8	6.9	20.7	37.7	14.5	22.4
	Calcium superphosphate (P)	35.8	20.3	22.6	33.8	23.4	25.6
	Potassium sulfate (K)	36.3	19.2	24.9	34.9	20.9	25.0
	NPK	26.9	40.1	29.4	25.7	41.7	30.0
Untreated	Untreated	44.9	0.0	7.6	44.1	0.0	8..0
L.S.D. at 5%							
Organic		7.8		5.6	6.3		5.2
Mineral		5.2		3.2	4.6		3.0
Interaction		8.4		5.9	6.9		5.7

(1) Farm-yard manure at rate of 75 kg/tree. (2) N.P.K. at the rate of 1, 1.5, 0.5 kg/tree, respectively.

(3) Average number of infected clusters/tree. (4) Average number of fruits/ branch.

Table (5): Effect of bactericides and mineral fertilizers on severity of fire blight and fruit production of pear during 2001 and 2002 growing seasons, at Toukh (Kalubia) region, under natural infection conditions.

Organic manure [1]	Mineral fertilizer [2]	Season 2001			Season 2002		
		Infection[3]	Disease Reduction (%)	Yield[4]	Infection[3]	Disease Reduction (%)	Yield[4]
Agrimycin 17	Untreated	22.4	50.1	33.7	21.1	52.2	35.4
	Ammonium nitrate (N)	21.0	53.2	35.0	19.9	54.9	36.4
	Calcium superphosphate (P)	15.0	66.6	37.7	14.3	67.6	38.3
	Potassium sulfate (K)	16.0	64.4	36.8	15.0	66.0	38.1
	NPK	10.2	77.3	41.1	9.8	77.8	72.9
Kocide 101	Untreated	28.7	36.1	29.9	26.7	41.8	33.2
	Ammonium nitrate (N)	26.4	41.2	29.8	24.3	44.9	34.3
	Calcium superphosphate (P)	20.1	55.2	31.4	19.8	55.1	35.6
	Potassium sulfate (K)	21.9	51.2	31.0	21.9	52.6	35.9
	NPK	16.7	62.8	36.1	15.9	63.9	38.7
Starner	Untreated	24.6	45.2	32.6	22.8	48.3	34.5
	Ammonium nitrate (N)	22.0	51.0	34.4	20.9	52.6	35.0
	Calcium superphosphate (P)	18.2	59.5	36.2	15.5	64.9	36.6
	Potassium sulfate (K)	19.4	56.8	36.0	16.3	63.0	35.3
	NPK	13.2	70.6	38.7	11.0	75.1	40.8
Untreated	Untreated	44.9	0.0	7.6	44.1	0.0	8.0
L.S.D. at 5%							
Organic		6.2		4.9	6.0		4.8
Mineral		5.6		4.5	5.3		4.7
Interaction		7.3		5.2	7.0		5.0

(1) Using at the rates of 0.5, 1.5, 1.5 g/l., respectively. (2) N.P.K. at the rate of 1, 1.5, 0.5 kg/tree, respectively.

(3) Average number of infected clusters/tree. (4) Average number of fruits/ branch.

Table (6): Effect of organic manure, mineral fertilizers and bactericides on severity of fire blight and fruit production of pear during 2001 and 2002 growing seasons, at Toukh (Kalubia) region, under natural infection conditions.

Organic manure [1]	Mineral fertilizer[2]	Bactericides[3]	Season 2001			Season 2002		
			Infection[4]	Disease Reduction (%)	Yield[5]	Infection[4]	Disease Reduction (%)	Yield[5]
Treated	NPK	Agrimycin 17	7.6	83.1	46.3	6.8	84.6	48.6
		Kocide 101	12.4	72.4	38.9	10.9	75.3	41.0
		Starner	9.6	78.6	40.6	8.6	81.5	47.4
Untreated	NPK	Agrimycin 17	10.2	77.5	41.1	9.8	77.8	42.9
		Kocide 101	16.7	62.8	36.1	15.9	63.9	38.7
		Starner	13.2	70.6	38.7	11.0	75.1	40.8
Untreated	Untreated	Agrimycin 17	22.4	50.1	33.7	21.1	52.2	35.4
		Kocide 101	28.7	36.1	29.7	26.7	41.3	33.2
		Starner	24.6	45.2	32.6	55.8	43.3	34.5
Untreated	Untreated	Untreated	44.9	0.0	7.6	44.1	0.0	8.0
L.S.D. at 5%								
Organic			3.7		4.3	3.5		4.4
Mineral			3.7		4.3	3.5		4.4
bactericide			2.9		3.8	2.7		3.9
Interaction			4.3		4.9	4.0		4.9

(1) Farm-yard manure at rate of 75 kg/tree.

(2)N.P.K. at the rate of 1, 1.5, 0.5 kg/tree, respectively.

(4)Average number of infected clusters/tree.

(5) Average number of fruits/ branch.

(5)Using at the rates of 0.5, 1.5, 1.5 g/l., respectively.

DISCUSSION

Symptoms, colony morphology on selective media, pathogenicity tests, Biolog System and serological assays all provided evidence to confirm the existence of fire blight in Egypt and the fact that *E. amylovora* is the pathogenic agent. It is possible that during 1964-1983 fire blight increased and spread near the center of its introduction, under Egyptian climatolcgical conditions in which rainfall occurs during March and April and average temperature during bloom of Le Conte vary from 9-26°C. In addition wind storms during April (bloom period), all these environments are generally favorable for the development and dispersal of the disease which become widespread in a large area of Behera, Menufia, Nubaria and Kalubia provinces and now in most Nile Delta of Egypt. Therefore, the disease varies in severity from year to year and from site to site because of variation in environmental conditions and differences in amount of initial inoculum.

In order to develop a management strategy to control fir blight an integrated approach was adopted using organic manure, mineral fertilizers and bactericides. Field experiments were conducted using Le Conte pear cultivar during 2000 and 2001 growing season at Kalubia governorate. The integration between organic manure and NPK were more effective than organic or NPK alone for reducing the disease incidence. Mean time the

application of Starner or Agrimycin during blooming period at three times 25%, 75%, and 100% of blooming was suggested for minimizing fire blight infection. The highest yield was attained as a result of using organic and mineral fertilizers integrated with bactericides. These results are in agreement with that obtained by Tawfik *et al.* (2002). Who noticed that the control of the disease depends on horticulture practices which minimize the blossom infection by early flowering and spray the blossoms with Starner, Agrimycin, or Kasugamycin. Also combination between bactericide and organic or mineral fertilizers treatment clearly reduced the disease severity and increased the yield (El-Zayat *et al.*, 1995). It is important to notice that Parker *et al.* (1961) found that combination between nitrogen and potassium or phosphorus fertilizers led to increase tree growth and a concomitant increase resistance to fire blight. Also Zwet and Beer (1995) reported that fertilization program could be designed to provide the proper balance of nutrients.

According to the aforementioned results, it can be stated that the strategies for fire blight control depends on:

1. Protecting the blossom by treatment with bactericides during blooming period.
2. Management of organic and mineral fertilizers to reduce susceptibility.
3. Reducing the efficacy and distribution of primary inoculum with copper compounds during dormancy.
4. Studying the environments.

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موقف مرض اللفحة النارية فى مصر

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يعتبر مرض اللفحة النارية مشكلة خطيرة ومرض مدمر لبساتين الكمثرى فى مصر وقد لوحظ المرض للمرة الأولى فى منطقة معمل القزاز والتى تقع فى المسافة بين الإسكندرية ودمنهور وذلك فى عام ١٩٦٤ وقد انتشر المرض بصورة وبائية فى مركز كفر الدوار بمحافظة البحيرة عام ١٩٨٢ وقد انتشر المرض بسرعة فى معظم المحافظات مما أدى إلى استئصال العديد من البساتين المصابة. أمكن فى هذه الدراسة تعريف ٢٣ عزلة باستعمال الاختبارات البكتريولوجية والطرق السيرولوجية (الارتباط المناعى على ورق النيتروسليولوز، الوميض الفلورسنتى للخلايا البكتيرية). وقد تم الحصول على تفاعلات إيجابية من أنسجة الأفرع المصابة لأشجار الكمثرى التى تظهر عليها الأعراض النموذجية للفحة النارية وذلك باستعمال D.I.A. ، I.F. وذلك عند استعمال السيرم المضاد المتخصص للمسبب المرضى ايروينيا أميلوفورا.

جميع المركبات الكيماوية المختبرة وكذلك المضادات الحيوية قللت من نمو البكتريا وشدة المرض على ثمار الكمثرى الغير ناضجة (العقد الحديث). وقد وجد أن مادة الاستارنر ٢٠% (اوكسولينيك اسيد) وحمض ناليدكسيسك ٢٠% من أكثر المركبات فاعلية فى الحد من نمو البكتريا تحت ظروف المعمل وكذلك التقليل من شدة الإصابة تحت ظروف الحقل. ومن ناحية أخرى فإن كوسيد ١٠١، كوسيد ٢٠٠٠ وأوكسى كلورو النحاس ٥٠% وأجريميسين ١٧ (كبريتات الاستربتوميسين) والابروميسين ٢٠% كانت كلها متوسطة الفاعلية أما كوسوجاميسين فكان الأقل فاعلية.

أجريت تجربة حقلية باستعمال صنف الكمثرى "لي كونت" خلال عامى ٢٠٠٢/٢٠٠١ بمحافظة القليوبية أمكن فى هذه التجربة باستعمال السماد العضوى والمعدنى N.P.K. (تسميد أزوتى – فوسفاتى – بوتاسى) والمضاد الحيوى اجريميسين ١٧ أو مركب استارنر ٢٠% التقليل بدرجة كبيرة من عدد العناقيد المصابة وتحقيق زيادة معنوية فى محصول الكمثرى بالمقارنة بالغير معامل ولا توجد فروق معنوية بين مركبات النحاس التى استخدمت فى الرش أثناء طور السكون. لذلك فقد أمكن وضع استراتيجية متكاملة لمقاومة هذا المرض باستعمال السماد العضوى والسماد المعدنى والمركبات المضادة للبكتريا رشا أثناء فترة التزهير.