

UNIVERSITY OF JORDAN  
FACULTY OF GRADUATE STUDIES

COMMON BLIGHT OF BEANS IN JORDAN VALLEY

BY

NASER SAMEH QADOUS

ADVISOR

Dr. HAMID M. KHLAIF

*Handwritten signature and Arabic text:*  
أستاذة الدكتور خالد  
[Signature]

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIRMENTS FOR THE  
DEGREE OF

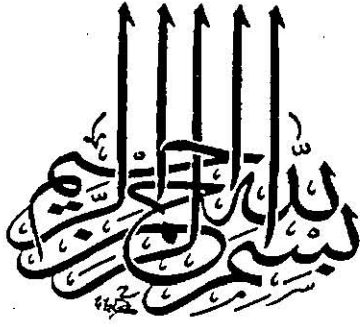
MASTER OF SCIENCE

IN

PLANT PROTECTION / PLANT PATHOLOGY

FACULTY OF GRADUATE STUDIES / UNIVERSITY OF JORDAN

MAY, 1993



**TO WHOM I LOVE**

**ACKNOWLEDGEMENT**

I would like to express my sincere gratitude to my supervisor Dr. Hamid khlaif for his advice, assistance, and inspiring guidance. Without his support and encouregment completion of this piece of research would have been impossible. I am also grateful to my teachers in Plant Protection Department, especially members of the defence committee : Professor N. Sharaf, Dr. H. Abu-Blan, and Dr.A. Mansour for their illuminating suggestions.

I should also express my gratefulness to my father, mother, sisters, and brothers, who all supported me until this study has been completed. Special thanks for uncle Abd-Alla Qatouni family for their help and encouregment.

Great thanks for Mr. Faysal Shraim and Zakaria Al-Sayed who offered their continuous help. Thanks for all my friends and colleagues especially Firas Abu El-Samen.



## TABLE OF CONTENTS

	PAGE
COMMITTEE DECISION .....	II
ACKNOWLEDGMENT.....	III
TABLE OF CONTENTS.....	IV
LIST OF TABLES.....	VII
LIST OF FIGURES.....	IX
LIST OF APPENDICES.....	XI
ABSTRACT.....	XII
1: INTRODUCTION.....	1
2: LITERATURE REVIEW.....	2
2:1: Survival of common blight pathogen...	4
2:1:1: Seeds.....	5
2:1:2: Plant debris.....	6
2:1:3: Survival as epiphytes.....	6
2:1:4: Role of soil.....	7
2:2: Control of common blight.....	7
3: MATERIALS AND METHODS.....	9
3:1: Isolation of the causal agent.....	9
3:2: Identification of the causal agent...	9
3:2:1: Oxidase test.....	10
3:2:2: Oxidative fermentative test.....	10
3:2:3: Yellow mucoid colonies on YDC.....	11
3:2:4: Mucoid colonies on NA+5% sucrose.....	11
3:2:5: Catalase test.....	11
3:2:6: Potatosoft rot.....	11
3:2:7: Starch hydrolysis.....	12
3:2:8: Gelatin hydrolysis.....	12

3:2:9: Pathogenicity test.....	12
3:3: Disease development and response of bean cultivars to the disease.....	13
3:4: Disease measurments.....	14
3:3:1: Disease incidence.....	14
3:3:2: Disease severity.....	15
3:5: Meteorological data.....	15
3:6: Statistical analysis.....	15
3:7: Response of bean cultivars to common blight.....	15
3:8: Disease development.....	16
3:9: Sources of inoculum.....	16
3:9:1: Seeds.....	16
3:9:2: Plant debris.....	18
3:9:3: Soil.....	19
3:9:4: Isolation from other hosts.....	20
3:10: Chemical control of common blight....	20
<b>4: RESULTS.....</b>	<b>23</b>
4:1: Isolation of the causal agent.....	23
4:2: Identification of the causal agent...	23
4:3: Pathogenicity test .....	23
4:4: Development of symptoms.....	28
4:5: Disease development.....	28
4:6: Response of tested bean cultivars to common blight.....	38
4:7: Sources of inoculum.....	44
4:7:1: Seeds.....	44
4:7:2: Plant debris.....	45
4:7:3: Soil.....	45
4:7:4: Other hosts.....	47

4:8: Chemical control of common blight....	47
5: DISCUSSION.....	51
6: CONCLUSIONS.....	55
7: REFERENCES.....	56
8: APPENDICES.....	62
9: ABSTRACT IN ARABIC .....	68

## LIST OF TABLES

TABLE	PAGE
1. Common names, chemical composition, and rates of applications of the tested compounds against common blight.....	22
2. Source, and results of laboratory and greenhouse experiments for Xcp collected from different bean growing areas in Jordan. ....	24
3. Average common blight incidence, severity, and category of susceptibility of the tested bean cultivars in experiment no 1.....	42
4. Average common blight incidence, severity, and category of susceptibility of the tested bean cultivars in experiment no 2.....	43
5. Average common blight incidence, severity, and category of susceptibility of the tested bean cultivars in experiment no. 3.....	43
6. Detection of Xcp from bean seeds by direct isolation on NA medium, and reisolation from bean seedlings inoculated with the seed soak.....	46
7. Detection and population of Xcp in bean debris, on MXP plates, and reisolation from bean seedlings inoculated with debris soak.....	46

8. Detection of Xcp from soil samples collected from different areas in the Jordan Valley, at monthly intervals..... 49
9. Average incidence, severity, and yield of the four replicates of the different treatments..... 50

## LISTS OF FIGURES

FIGURE	PAGE
1. Some biochemical tests used in the identification of <i>Xanthomonas campestris</i> pv. <i>phaseoli</i> .....	26
A. Oxidative fermentative test	
B. Mucoïd colonies on NA+5% sucrose medium.	
C. Yellow mucoïd colonies on yeast extract dextrose calcium carbonate agar medium.	
D. Starch hydrolysis.	
E. Gelatin hydrolysis	
F. Potato soft rot	
2. Artificially inoculated bean seedling showing positive reaction for <i>Xanthomonas campestris</i> pv. <i>phaseoli</i> .....	29
3. Symptoms of common blight of beans on :.....	30
A. Leaves	
B. Pods	
C. Seeds	
4. Symptoms of <i>Xanthomonas campestris</i> pv. <i>phaseoli</i> on :	
A. <i>Ipomoea purpurea</i>	31
B. <i>Malva syriaca</i> .....	
C. <i>Vigna unguiculata</i>	
d. <i>Vigna luteola</i> .....	32
5. Development of common blight on tested bean cultivars during fall, 1991 . (Experiment no.1)..	34
6. Average temperature in the open field for experiment no.1, fall, 1991. ....	35
7. Average relative humidity in the open field for experiment no. 1.....	35

### Abstract

Fourty two different bacterial isolates, obtained from diseased bean plants collected from different growing areas in Jordan Valley, were biochemically and physiologically tested for common blight disease. Results indicated that the causal organism of common blight is [*Xanthomonas campestris* pv. *phaseoli* (Smith) dye]. (Xcp).

Three experiments were carried out at the University Research Station in Central Jordan Valley to study development of common blight in relation to temperature and relative humidity and the response of eight bean cultivars to the diseases. The results indicated that : Common blight developed at temperatures ranging from 7.1 to 39.5 °C, and relative humidity between 30.6 and 85.1%. Optimal conditions for common blight were about 24 °C, and relative humidity more than 50 % or high rainfall. All tested cultivars were susceptible to common blight infection, but with various degrees. Kentucky Wonder and Trophy were found to be susceptible. Tema, Strike, and Wade were moderately resistant, while Contissa, Lollita, and Nerina R.S. varried in response, from moderately resistant to moderately susceptible depending on planting date.

Pathogenic strains of Xcp were isolated from the soak of all tested bean seed samples, from bean debris

throughout the period in which Jordan Valley is free from cultivated bean, and also from diseased leaves of the following plants *Ipomoea purpurea* L., *Malva syriaca* L., *Pisum sativum* L., *Vigna luteola* (Jacq.) Benth., and *Vigna unguiculata* (L.) Walp. These plants could be considered as new hosts for common blight, and might bring a new dimension to the epidemiology of the disease. So seeds, bean debris, and other hosts could be considered as major sources of inoculum for common blight in Jordan Valley, but not soil.

All tested compounds : Tri-Miltox, Cuprosan Dithane-M-45, Ditiver and Oxytetracycline, suppressed disease incidence and severity significantly and produced higher marketable yield compared to the check. Tri-Miltox, appeared to be the most effective compound.



## 1: Introduction

Green bean (*Phaseolus vulgaris* L.) is one of the major vegetable crops grown in Jordan. The area planted to beans in 1991 was (8504.3) dunums, with a total production of (6904.4) metric tons, (i.e) (812 Kg per dunum). Seventy eight percent of the area planted to beans is located in the Jordan Valley. (Dept. of General Statistics, 1991). Beans in Jordan are planted under plastichouses as well as in the open field.

The following bacterial diseases were reported to attack beans in Jordan; Common blight : [*Xanthomonas campestris* pv. *phaseoli* (Smith) Dye], hereafter referred to as (Xcp), brown spot [*Pseudomonas syringae* pv. *syringae*) Van Hall], halo blight [*Pseudomonas syringae* pv. *phaseolicola* (Burk.) Dows.] and bean stem knot [*Pseudomonas viridiflava* (Burk.) Clara.] (Abu Blan and Khlaif, 1993). Because the lack of information about common blight of bean in Jordan this research was conducted in order to :

- 1- Isolate and identify the causal agent.
- 2- Study the disease development in relation to temperature and relative humidity.
- 3- Test the response of different bean cultivars to the causal agent.
- 4- Investigate the possible sources of inoculum. And to
- 5- Test the effect of different chemicals on the development of common blight.

## **LITERATURE REVIEW**

## 2: Literature Review

Bacterial blights of beans : (common blight [*Xanthomonas campestris* pv. *phaseoli* (Smith) Dye.], halo blight [*Pseudomonas syringae* pv. *phaseolicola* (Burk.) Dow.], and brown spot [*Pseudomonas syringae* pv. *syringae* Van Hall]), are among the most important diseases attacking beans all over the world. These blights drastically affect the yield and can cause epidemics and losses exceeding 75-90% of the potential yield (Venette and Lamey, 1978).

Field diagnosis of common blight without further precise laboratory examination could be misleading due to close similarity in early symptoms developed by other mentioned bacterial blights, therefore it is necessary to characterize and identify the causal agent of common blight by biochemical and physiological tests (Webster, et al., 1983 A).

Common blight which is favoured by warm temperatures (28 °C) and humid conditions (Venette, 1978), still the major disease facing bean producers in the tropical and sub-tropical areas. While halo blight and brown spot are favoured by cool temperatures (16-21 °C) and rainy wet weather, (Hayward and Waterson, 1965).

Xcp is an aerobic, gram negative, rod-shaped, motile by a single polar flagellum, with oxidative

metabolism, it hydrolyses starch and gelatin, oxidase negative, catalase positive, potato soft negative; produces yellow mucoid colonies on yeast extract calcium carbonate agar (YDC) medium, and domed mucoid colonies on Nutrient Agar (NA) supplemented with 5% sucrose (Krieg and Holt, 1984).

The development and multiplication of Xcp in and on plant parts resemble growth curve of bacteria in-vitro, with a mean doubling time of 19.4 hours (Weller and Saettler, 1980 A). However, this pattern was found to be affected by external factors such as low temperature which increased the doubling time; and internal factors such as plant part, and host genotype (Weller and Saettler, 1980 A).

The appearance of symptoms require a minimum of  $5 \times 10^6$  bacterial cells per 20 cm<sup>2</sup> of plant canopy (Weller and Saettler, 1980 A).

Common blight disease attacks primary leaves, trifoliolate leaves, stems, pods and seeds (Aggour, et al. 1989 ; Weller and Saettler, 1980 A). Symptoms on leaves start as small, water-soaked spots. These spots enlarge and join together forming large necrotic areas surrounded by lemon yellow halo. The whole leaf collapse later.

Infected stems show small water-soaked and sunken lesions that gradually develop to elongated spots and turn brown (Weller and Saettler, 1980 B). The bacteria could move systemically through leaf pedicels to the stem resulting in red discoloration of leaf vein and stem tissues. Lesions may

الصفحة غير موجودة من أصل المصدر

weeds.

**2:1:1 Seeds :** Seeds are the major source of primary inoculum of common blight (Malin *et al.*, 1983; Saettler and Perry, 1976; Schaad, 1982; Weller and Saettler, 1980 B); and Yoshi *et al.*, 1978).

Most seed-borne bacteria survive as long as the seed remain viable (Schuster and Coyne, 1974). Fifteen-year survival period in seeds was reported for Xcp (Gilbertson *et al.* 1988). Xcp was reported to be found on the surface of seeds, internally beneath seed coat, but few bacteria were detected within cotyledons and embryo tissues (Khlaif, 1986), which brought a new dimension in the epidemiology of this disease.

Several methods were employed to detect Xcp in seeds **Of these :**

**1- Field inspection :** fields grown to beans are monitored for the appearance of disease symptoms and the percentage of infected plants are recorded. This test is not sensitive, since it depends on the accurate diagnosis of the disease symptoms in the field, which could be masked with the symptoms of other diseases. Symptom expression depends on environmental conditions and host genotype. Also low levels of infection are not detected. (Schaad, 1982).

**2- Growing on :** seeds are grown in the field or under the greenhouse conditions, away from any source of Xcp, and are monitored carefully for development of common blight symptoms (Ralph, 1979). This test is time consuming and

الصفحة غير موجودة من أصل المصدر

conditions (Leben, 1965). Resistant or volunteer symptomless bean plants were reported to harbor Xcp (Schuster and Coyne, 1974; Sharen, 1959). Also many non host crops and weeds harbor Xcp and act as source of inoculum for secondary disease spread. These plants are cowpea [*Vigna unguiculata* (L.) Walp], corn [*Zea mays* L.], soybean [*Glycine max* L.], Beet [*Beta vulgaris* L.], pigweed [*Amaranthus retroflexus* L.], and lambsquarters [*Chenopodium album* L.]. (Cafati and Saettler, 1980 B; Angeles-Ramos, et al. 1991).

**2:1:4: Survival of Xcp in soil :** Xcp was reported to survive as free cells for 3 months in field soil at 25 °C, and for 6-9 months at 5 °C (Graham, 1953), and for 2 weeks in the bean rhizosphere (Stanek and Lasik, 1965).

### **2:2: Control of Common Blight**

Survival of Xcp in seeds and plant debris, and its colonization of host and non host plants for a long period makes the control of common blight very difficult. However, different measures were employed to control the disease. **Of these:**

- (1) Planting bacteria free seeds, through certification programs. A zero tolerance is required for the certification program in Idaho and European and Mediterranean Plant Protection Organization countries (Lahman and Schaad, 1985; Webster, et al., 1983 A). While Michigan permits 0.005% blighted plants during field inspection, and no infected seeds in laboratory tests



(Copeland, et al., 1975).

(2) Two-year crop rotation, and destruction of bean debris by burning or deep plowing, as Xcp was reported to survive for shorter period in underground debris, where antagonistic microorganisms thrive (Gilbertson, et al., 1990).

(3) Resistant cultivars are the most important control strategy, even though high level of resistance is not available (Zapata, et al., 1985). Resistant genes are transferred from other wild *Phaseolus* spp. such as *P. acutifolius* and *P. coccineus*. However, all germplasm sources are systemically colonized by Xcp. (Dermot, et al. 1973).

(4) Chemical control : different copper-containing compounds were used to control bacterial diseases, but there were no satisfactory chemical control treatments for common blight (Saettler, 1971).

(5) Seed treatment with bactericides such as streptomycin resulted in decreasing surface contamination, but did not affect internal bacteria (Webster, et al. 1983 A).

## MATERIALS AND METHODS

### 3: Materials and Methods

#### 3:1: Isolation of the causal agent:

Bean samples ( leaves, stems, and pods) suspected to be infected with common blight were collected from different bean growing areas in Jordan Valley (Table 2).

Small portions from each sample were cut into small pieces with a sterile scalpel and soaked into a drop of sterile distilled water on a sterile slide. The resulted suspension was left to stand for 5 minutes.

A loopful from the suspension was streaked onto the surface of a dried nutrient agar (NA) plate with a sterile platinum loop. Plates were incubated at  $25\pm 2$  °C till bacterial growth started to develop, single yellow colonies were restreaked onto another NA plate to obtain pure cultures. These isolates were grown on NA test tubes and kept in a refrigerator for further identification (Gerhardt et al., 1981; Lelliot and Stead, 1987; Schaad, 1988).

424301

#### 3:2: Identification of the causal agent

Thirty six-hour-old cultures of the obtained isolates were subjected to laboratory tests for characterization and identification according to methods described by Schaad (1988). These tests were : oxidase, oxidative/fermentative, production of yellow mucoid colonies on YDC, production of mucoid colonies on NA+5% sucrose, catalase, starch hydrolysis, gelatin hydrolysis, and pathogenicity to bean seedlings.

**3:2:1. Oxidase test :**

This test aims to detect the presence of cytochrom C, and to differentiate between saprophytic *Xanthomonas* which have cytochrome C, and phytopathogenic ones which do not have it. (Gerhardt et al., 1981; Lelliot and Stead, 1987). A loopful of bacterial mass was spotted on a sterile filter paper, in a sterile Petri dish saturated with freshly prepared 1.0% (W/V) aqueous solution of tetramethyl-p-phenylenediamine dihydro-chloride (Sigma).

The observation of a dark blue color within 10 seconds was considered positive. The reactions were compared with the reactions of the reference bacterial culture of *Pseudomonas aeruginosa* (ATCC 10145) as a positive check (Gerhardt, et al., 1981).

**3:2:2 Oxidative/Fermentative (OF) test**

To test the ability of a bacterium to utilize glucose oxidatively or fermentatively, a loopful of the bacterial growth was stab inoculated into the semi-solid OF-basal medium (peptone 2.0g, NaCl 5.0g, dipotassium phosphate ( $K_2 HPO_4$ ) 0.3g, bromothymol blue 0.03g, Bacto agar 3.0g, and 1.0% dextrose added to 1L distilled water). Inoculated tubes were covered with a layer of sterile 2.0% molten agar and incubated at  $25 \pm 2$  °C for 24-48 hrs. Change in color from dark green to yellow was considered positive reaction (Fig. 1A (page 26 )) Gerhardt, et al., 1981; Schaad, 1988). The reference culture of the bacterium *Erwinia carotovora* pv. *carotovora* (ATCC 15713) was used as a positive control.

**3:2:3: Yellow mucoid colonies on YDC :**

Xanthomonads produce large, smooth, domed and mucoid yellow colonies on YDC (yeast extract 10.0g, dextrose 20.0g, calcium carbonate (light powder) 20.0g, Bacto agar (Difco) 15.0 in 1L distilled water) (Schaad, 1988; Lelliot and Stead, 1987).

A loopful of bacterial growth was spot inoculated onto the surface of a YDC plate, plates were incubated for 48 hrs. The formation of a dome of yellow mucoid colonies was considered as positive, (Fig. 1B (page 26)).

**3:2:4: Mucoid colonies on NA+5% sucrose:**

A loopful of the tested bacterium was spot inoculated on a NA plate supplemented with 5% sucrose, and treated as in the previous test. Formation of domed, mucoid colonies was considered as positive test (Fig. 1C (page 26)). (Gerhardt, et al., 1981; Lelliot and Stead, 1987; Schaad, 1988).

**3:2:5: Catalase test :**

A loopful of bacteria was added to a drop of 3.0% aqueous solution of hydrogen peroxide ( $H_2O_2$ ). Bubble formation indicated the presence of catalase enzyme and bacteria that formed bubbles were considered as positive. (Gerhardt, et al., 1981).

**3:2:6: Potato soft rot :**

The purpose of this test is to detect pectolytic activity of the tested bacterium. A "V"-shaped groove was cut in a potato slice made with a sterile scalpel, and inoculated by a sterile platinum loop with the bacteria.

Slices were incubated at  $25 \pm 2$  °C in a moistened Petri plate for 24-48 hrs. Soft rotting of potato tissue was

considered as positive result (Fig. 1D (page 27)).

The reference culture of the bacterium *Erwinia carotovora* pv. *carotovora* (ATCC 15713) was used for positive check, and slices inoculated with sterile distilled water were used to serve as a negative control (Lelliot and Stead, 1987; Schaad, 1988).

### **3:2:7: Starch hydrolysis test :**

The purpose of this test is to detect the presence of amylase enzyme.

A loopful of the tested bacterium was spot inoculated onto a NA plate supplemented with 0.2% potato soluble starch. Plates were incubated at  $25 \pm 2$  °C for 48-72 hrs, then were flooded with Lugol's iodine. Formation of clear zone around the colony was considered as a positive reaction, (Fig. 1E (page 27)) (Gerhardt, et al, 1981).

### **3:2:8: Gelatin hydrolysis :**

A loopful of the bacterial growth was spot inoculated onto a NA plate plus 0.4% gelatin in a Petri plate. Plates were incubated at  $25 \pm 2$  °C for 48-72 hrs. These plates were then flooded with 5-10 ml of acid mercuric chloride solution ( $\text{HgCl}_2$  , 15g; distilled water, 80 ml; concentrated HCl (33%), 16ml). Formation of a clear zone around the colonies was considered as a positive reaction, which indicates the presence of gelatinase enzyme, (Fig. 1F (page 27)) Schaad, 1988).

### **3:2:9 Pathogenicity test :**

Pathogenicity tests for the obtained isolates were conducted according to the methods described by Lelliot and Stead (1987), and Schaad (1988).  $10^6$ - $10^7$  colony

forming unit (CFU)/ml bacterial suspension prepared from 36-hr-old cultures from each isolate was inoculated by an artist air brush under high pressure (15-20 psi) to the lower surface of primary leaves of four bean seedlings 2-weeks-old of Trophy and/ or Kentucky Wonder cultivars.

A second group consisting of other four bean seedlings were inoculated with sterile distilled water to serve as a control. A third group of four bean seedlings, were inoculated with a bacterial suspension of the reference culture of Xcp (ATCC 9563).

Inoculated plants were kept at  $25 \pm 2$  °C under mist chamber (100% RH) for 48 hrs, then these plants were placed on a greenhouse bench till symptoms started to develop (Fig. 2 (page29)).

All the above mentioned tests were run on the reference culture of Xcp (ATCC 9563) to serve as a check.

### **3:3: Disease development and response of bean cultivars to common blight :**

Three field experiments were conducted at the University Research Station, Central Jordan Valley. The first and third experiments were carried out in the open field, whereas the second experiment was carried out under the plastic house.

The three experiments were initiated in the following dates : October 1st, 1991, November, 26th, 1991, and February, 20th, 1992, for experiments 1, 2, and 3, respectively.

The designs of the three experiments were identical

and conducted in the same manner as follows : Eight bean cultivars were used in the three experiments, these were : Kentucky Wonder and Lollita as climbing bean cultivars, and Contissa, Nerina RS, Strike, Tema, Trophy, and Wade as bushy cultivars. The layout of each experiment was a randomized complete block design. Seeds were planted in rows, 1m apart, and a distance of 30 cm between seeds. Two lines were planted in each row. Each cultivar (treatment) was replicated four times, each plot consisted of 6m-long row. Two weeks later, after the emergence of the seedlings plants were drenched with a mixture of Benlate<sup>R</sup> (1.0 g/L), and Tachigreen<sup>R</sup> (1.5 g/L) to protect them from wilt and damping-off diseases. All plants were sprayed with Phosdrin<sup>R</sup> (1.0 ml/L) and/or Dursban<sup>R</sup> (2.0 ml/L), Kelthion<sup>R</sup> (2.0 ml/L), and Benlate<sup>R</sup> (0.75g/L) to control insect, mite infestation, and fungal infections, respectively, as needed. Plants were fertilized by applying Engress soluble<sup>R</sup> (40:20:20 + trace elements) fertilizer in the drip system.

### **3:4: Disease measurements :**

The incidence and severity of natural infection of common blight were recorded at weekly intervals in the three experiments starting when the first disease signs were observed and confirmed by laboratory tests .

#### **3:4:1: Disease Incidence :**

Twenty plants were chosen alternatively in each plot and labeled before the appearance of symptoms. The incidence was calculated as the percentage of infected leaves out of the total leaves of each of the labelled



plants. Any leaf showing just one spot was considered as infected. The average incidence for the four replicates for each cultivar was calculated.

### **3:4:2: Disease Severity :**

Twenty leaves were taken randomly from each plot and rated according to Horsfall and Heuberger (1942) numerical scale, as follows :

- 0 = no symptoms
- 1 = 1-10% of the leaf area covered with spot.
- 2 = 11-25% of the leaf area covered with spot.
- 3 = 26-50% of the leaf area covered with spot.
- 4 = 51-75% of the leaf area covered with spot.
- 5 > 75% of the leaf area covered with spot.

### **3:5: Meteorological Data**

Data on temperature and relative humidity for experiments 1 and 3 were taken from the meteorological station at the University Research Station, but for plastic house experiment temperature and relative humidity were recorded by a thermohydrograph placed in the middle of the experiment. Averages of minimum, and maximum of temperature and relative humidity were calculated weekly.

### **3:6: Statistical analysis :**

Data on incidence and severity of the disease were statistically analyzed. Significantly different means were separated by the least significant difference test (L.S.D. at  $p=0.05$ ).

### **3:7: Response of bean cultivars to common blight :**

The average incidence of the four replicates for each cultivar was calculated in each experiment. The responses

of the eight bean cultivars to the disease were evaluated according to Peterson, et al., (1948) scale as follows :

<u>Incidence range</u>	<u>Infection type</u>	<u>Symbol</u>
zero	Immune	I
1-10%	Resistant	R
11-25%	Moderately resistant	MR
26-50%	Moderately Susceptible	MS
> 50%	Susceptible	S

### **3:8: Disease Development:**

Average incidences of the four replicates of each cultivar were calculated weekly during the growing season, then plotted against average minimum, mean, and maximum of temperature and relative humidity during the experimental periods.

### **3:9: Sources of inoculum:**

#### **3:9:1: Seeds :**

Seeds of the eight bean cultivars were assayed for the presence of the pathogen. A random sample of 100-grams of seeds of each cultivar was surface sterilized with 0.5% sodium hypochlorite, rinsed three times in sterile distilled water, and then incubated in a flask containing 250 ml of sterile enrichment medium (yeast extract, 10g; crystal violet, 1ml of 0.075% in 1L of

phosphate buffer saline (PBS) (0.01M, pH 7.2)) (Khlaif, 1986). Flasks were placed on a rotary shaker (200 rpm) at room temperature for 36 hrs, then seed soaks were collected and the following procedures were performed :-

1- A loopful of each suspension was streaked onto the surface of a NA dried plate. Plates were incubated at  $25 \pm 2$  °C until bacterial growth developed. Four yellow colonies from each plate were subcultured onto new NA plates. Plates were incubated at  $25 \pm 2$  °C until bacterial growth developed. Then these isolates were biochemically and pathologically tested for the presence of the pathogenic Xcp.

2- A portion of the same seed soak of each bean cultivar was infiltrated under high pressure (20 psi), using an artist air brush, into the lower surface of primary leaves of four, 2-weeks-old seedlings of the same cultivar from which the soak was produced. Another four seedlings from each bean cultivar were infiltrated with sterile enrichment medium only, and a third set of four seedlings were infiltrated by sterile distilled water. And yet another set of 4 seedlings were inoculated with a suspension of the reference bacterial culture (Xcp ATCC 9563) to serve as check to compare the developing symptoms.

Inoculated seedlings were kept under a mist chamber for 48 hrs, then were placed on a greenhouse bench at  $25 \pm 2$  °C until symptoms of the disease developed. Then a portion of the leaves that showed symptoms similar to those induced by the reference strain were taken aseptically and placed in a drop of sterile distilled water, cut into

small pieces and left to stand for 5 minutes. A loopful of the suspension was streaked onto the surface of a dried NA plate. The isolates were identified as described earlier.

### 3:9:2: Plant Debris :

Samples of bean plant debris known to be infected with common blight were collected randomly from the field at monthly intervals, starting early June, until early November. A portion of each sample was ground in a Waring Blender. One gram of the ground debris was suspended into a flask containing 100 ml of sterile phosphate buffer (0.01 M, pH 7.2). Flasks were placed on a rotary shaker for 2 hrs (Gilbertson, 1990; Lelliot and Stead, 1987). Then the following procedures were performed:

1. Ten-fold serial dilutions were prepared from each suspension, then 100  $\mu$ l of  $10^{-4}$ ,  $10^{-6}$ , and  $10^{-8}$  dilutions were taken and spread by means of sterile L-shaped glass rod onto the surface of 4 plates of MXP, a semi-selective medium for isolation of Xcp, it contains (in grams per liter): [ $K_2 HPO_4$  (0.8),  $KH_2 PO_4$  (0.6), yeast extract (0.7), soluble potato starch (8.0), potassium bromide (10.00), glucose (1.0), and agar (15.0). After autoclaving, chlorothalonil (15.0 mg/L), ceplalixin (20)mg/L), kasugamycin (20 mg/L), gentamycin (2.0 mg/l), methylviolet 2B (30  $\mu$ l/L; 1% solution in 20% ethanol), and methyl green (60  $\mu$ l/L; 1% aqueous solution) were added]. (Claflin, 1987). Plates were incubated at  $25 \pm 2$  °C until bacterial growth developed, colonies surrounded by clear zone were counted, then CFU/ml were calculated. Representative colonies from

each plate were subcultured onto NA plates, and subjected for biochemical and pathogenicity tests for characterization and identification to test if the pathogen was isolated.

2. Another portion of the debris suspension was used for the inoculation of primary leaves of eight 2-weeks-old bean seedlings from Trophy and Kentucky Wonder cultivars, (4 from each one), by an artist air brush under high pressure (20 psi). In addition, a set of 8 seedlings were inoculated with sterile PB to serve as a check. Also a third set of 8 seedlings were inoculated with reference strain of Xcp (ATCC 9563), to compare the developed symptoms.

All inoculated seedlings were kept under mist chamber, (100% RH) for 2-3 days and then placed on a greenhouse bench, and checked daily for symptoms development.

Small portions of leaves showing typical symptoms of common blight were taken, then isolation and identification of the pathogen was done as previously described.

### 3:9:3: Soil :

Different fields that were known to be planted with beans infected with common blight last season were chosen in the Jordan Valley (Table 8) .

Composite soil samples of 500 g were taken randomly from the top 15 cm surface soil at monthly intervals starting from early May until end of December of the same year.

الصفحة غير موجودة من أصل المصدر

the manufacturers, (Table 1).

The highly susceptible Trophy bean cultivar, as a result from experiment no.1, was used in this experiment.

The experiment was conducted on February 20, 1992. The layout of the experiment was a randomized complete block design. Seeds were planted in rows 5 meters long, 1.8 m apart, at a distance of 30 cm between seeds. Each treatment was replicated 4 times.

Spraying of chemicals at 10-days interval, started at the 3 trifoliate leaf stage, until pod maturation.

Incidence and severity of the disease was measured as previously mentioned. Total marketable yield and average production of each treatment were calculated.

Data on incidence, severity and yield (total marketable) were statistically analyzed, and significantly different means were separated by L.S.D. test at ( $p=0.05$ ) level (Table 9).

Table (1) : Common names, chemical composition, and concentrations of the tested compounds against common blight in the field.

Trade Name	Chemical Composition	Conc g/L
Tri-Miltox forte	Mancozeb, 20%; Pure Copper (Oxy-chloride, carbonate and sulfate, 21.5	2.0
Ditiver(double)	Copper oxychloride, 37.5%; Zenib, 15%	2.5
Cuprosan 311 Super D	Cuprosan, 30%; Copper oxychloride, 52%; Zenib, 10%; Maneb,	2.5
Diathane-M-45 (Mancozeb)	Zenib, 16%; Maneb, 2%; Ethylene bis di-thiocarbamate 62%.	2.5
Oxytetracycline 25%	Oxytetracycline HCl 250g/Kg	1.0



## **4: Results**

### **4:1 Isolation of the causal agent**

Small, yellow, smooth bacterial colonies developed after 24-36 hrs of incubation onto NA plates, streaked with the suspensions of the naturally infected parts of bean plants.

### **4:2: Identification of the causal agent:**

The reaction of obtained isolates to the different biochemical and nutritional tests are presented in (Table 2).

All isolates were oxidase negative, oxidative metabolism of glucose, catalase positive, yellow mucoid and domed colonies were produced on NA+ 5% sucrose medium, and YDC medium, positive hydrolysis of starch and gelatin, and potato soft rot negative (Fig. 1).

### **4:3: Pathogenicity test :**

All the tested isolates showed positive reaction on leaves of Trophy and/or Kentucky Wonder bean seedlings (Table 2). The reactions were similar to those of the reference culture of Xcp (ATCC 9563). Two days after inoculation the inoculated area showed water soaking, enlarged in size, these spots became necrotic after 7-14 days surrounded with a lemon yellow halo, (Fig. 2). Seedlings inoculated with sterile distilled water didn't show any reaction.

TABLE (2): Source, and results of laboratory and green house tests for *Xanthomonas campestris* pv. *phaseoli* collected from different bean growing areas in the Jordan Valley.

ISOLATE	ORIGIN	BIOCHEMICAL TESTS *								PATH.
		O	OF	C	L	YDC	SH	GH	PSR	
Xcp 1	N. Shouneh	-	-	+	+	+	+	+	-	+
Xcp 2	N. Shouneh	-	-	+	+	+	+	+	-	+
Xcp 3	N. Shouneh	-	-	+	+	+	+	+	-	+
Xcp 4	N. Shouneh	-	-	+	+	+	+	+	-	+
Xcp 5	N. Shouneh	-	-	+	+	+	+	+	-	+
Xcp 6	Wadi Elyabis	-	-	+	+	+	+	+	-	+
Xcp 7	Wadi Elyabis	-	-	+	+	+	+	+	-	+
Xcp 8	Wadi Elyabis	-	-	+	+	+	+	+	-	+
Xcp 9	Wadi Elyabis	-	-	+	+	+	+	+	-	+
Xcp 10	Wadi Elyabis	-	-	+	+	+	+	+	-	+
Xcp 11	Deir-Alla	-	-	+	+	+	+	+	-	+
Xcp 12	Deir-Alla	-	-	+	+	+	+	+	-	+
Xcp 13	Deir-Alla	-	-	+	+	+	+	+	-	+
Xcp 14	Deir-Alla	-	-	+	+	+	+	+	-	+
Xcp 15	Deir-Alla	-	-	+	+	+	+	+	-	+
Xcp 16	Karameh	-	-	+	+	+	+	+	-	+
Xcp 17	Karameh	-	-	+	+	+	+	+	-	+
Xcp 18	Karameh	-	-	+	+	+	+	+	-	+
Xcp 19	Karameh	-	-	+	+	+	+	+	-	+
Xcp 20	Karameh	-	-	+	+	+	+	+	-	+
Xcp 21	S. Shouneh	-	-	+	+	+	+	+	-	+
Xcp 22	S. Shouneh	-	-	+	+	+	+	+	-	+
Xcp 23	S. Shouneh	-	-	+	+	+	+	+	-	+
Xcp 24	S. Shouneh	-	-	+	+	+	+	+	-	+
Xcp 25	S. Shouneh	-	-	+	+	+	+	+	-	+
Xcp 26	Wadi Shoaib	-	-	+	+	+	+	+	-	+
Xcp 27	Wadi Shoaib	-	-	+	+	+	+	+	-	+
Xcp 28	Wadi Shoaib	-	-	+	+	+	+	+	-	+
Xcp 29	Wadi Shoaib	-	-	+	+	+	+	+	-	+

TABLE (2) CONTINUED ...

Isolate	Origin	O	OF	C	L	YDC	SH	GH	PSR	PATH.
Xcp 30	Wadi Shoaib	-	-	+	+	+	+	+	-	+
Xcp 31	Bean Debris	-	-	+	+	+	+	+	-	+
Xcp 32	Bean Debris	-	-	+	+	+	+	+	-	+
Xcp 33	Bean Debris	-	-	+	+	+	+	+	-	+
Xcp 34	Bean Debris	-	-	+	+	+	+	+	-	+
Xcp 35	Bean Debris	-	-	+	+	+	+	+	-	+
Xcp 36	Seeds	-	-	+	+	+	+	+	-	+
Xcp 37	Seeds	-	-	+	+	+	+	+	-	+
Xcp 38	Seeds	-	-	+	+	+	+	+	-	+
Xcp 39	Seeds	-	-	+	+	+	+	+	-	+
Xcp 40	Seeds	-	-	+	+	+	+	+	-	+
Xcp 41	Seeds	-	-	+	+	+	+	+	-	+
Xcp 42	Seeds	-	-	+	+	+	+	+	-	+
Xcp 43	<i>V. unguiculata</i>	-	-	+	+	+	+	+	-	+
Xcp 44	<i>V. unguiculata</i>	-	-	+	+	+	+	+	-	+
Xcp 45	<i>I. purpurea</i>	-	-	+	+	+	+	+	-	+
Xcp 46	<i>I. purpurea</i>	-	-	+	+	+	+	+	-	+
Xcp 47	<i>M. syriaca</i>	-	-	+	+	+	+	+	-	+
Xcp 48	<i>M. syriaca</i>	-	-	+	+	+	+	+	-	+
Xcp 49	<i>P. sativum</i>	-	-	+	+	+	+	+	-	+
Xcp 50	<i>P. sativum</i>	-	-	+	+	+	+	+	-	+
Xcp 51	<i>V. luteola</i>	-	-	+	+	+	+	+	-	+
Xcp 52	<i>V. luteola</i>	-	-	+	+	+	+	+	-	+
ATCC 9563	Reference Culture	-	-	+	+	+	+	+	-	+

O = oxidase      OF = Oxidative Fermentative      C = Catalase

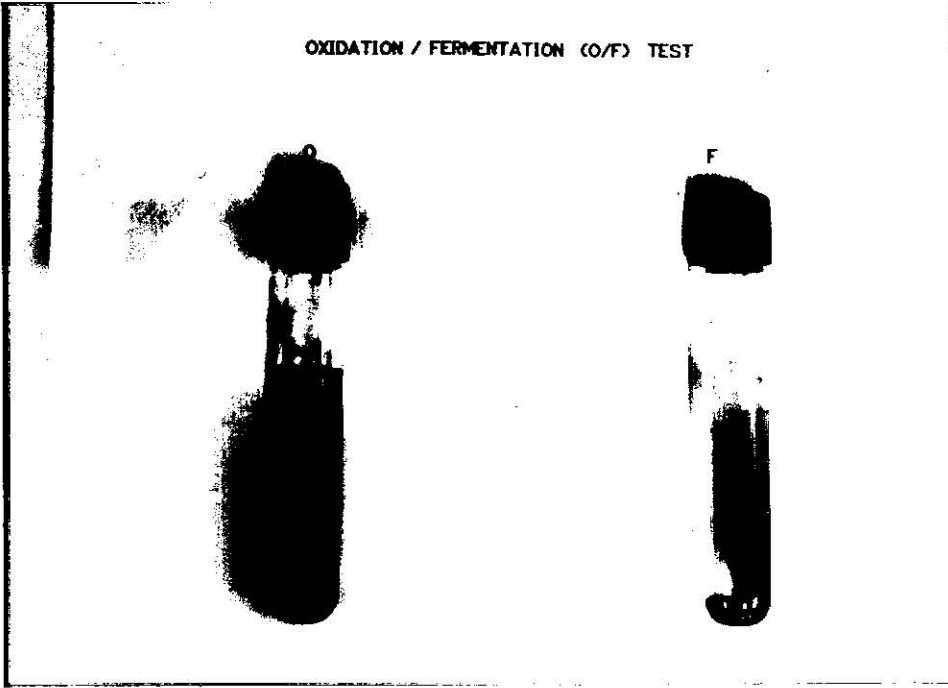
L = Mucoid Colonies on NA+5% Sucrose

YDC = Mucoid Colonies on Yeast Extract Calcium Carbonate Agar

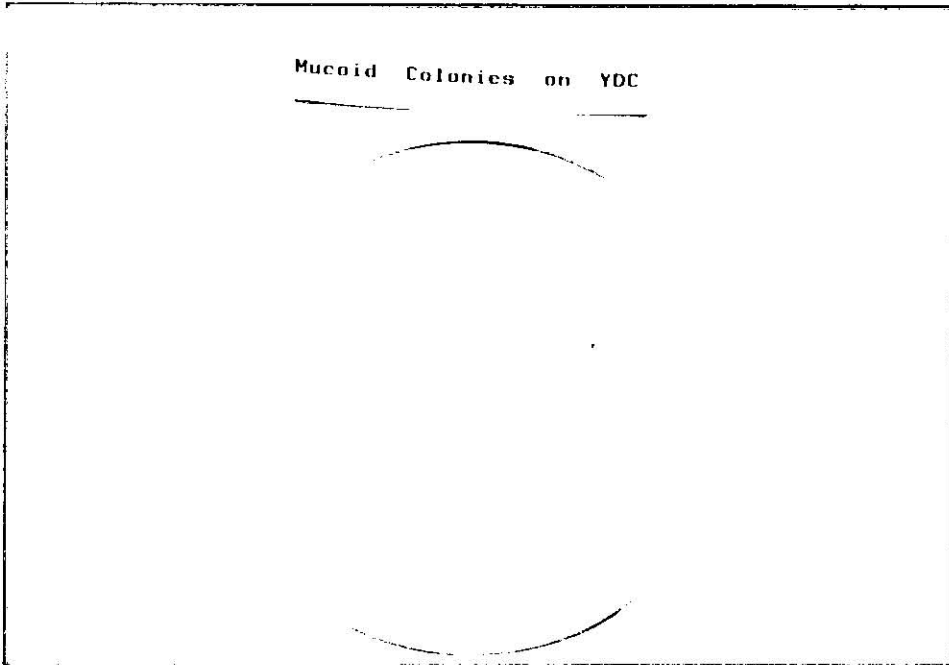
SH = Starch Hydrolysis      GH = Gelatin Hydrolysis

Path.= Pathogenicity to Trophy and/or Kentucky Wonder seedlings

A.



B.



C.

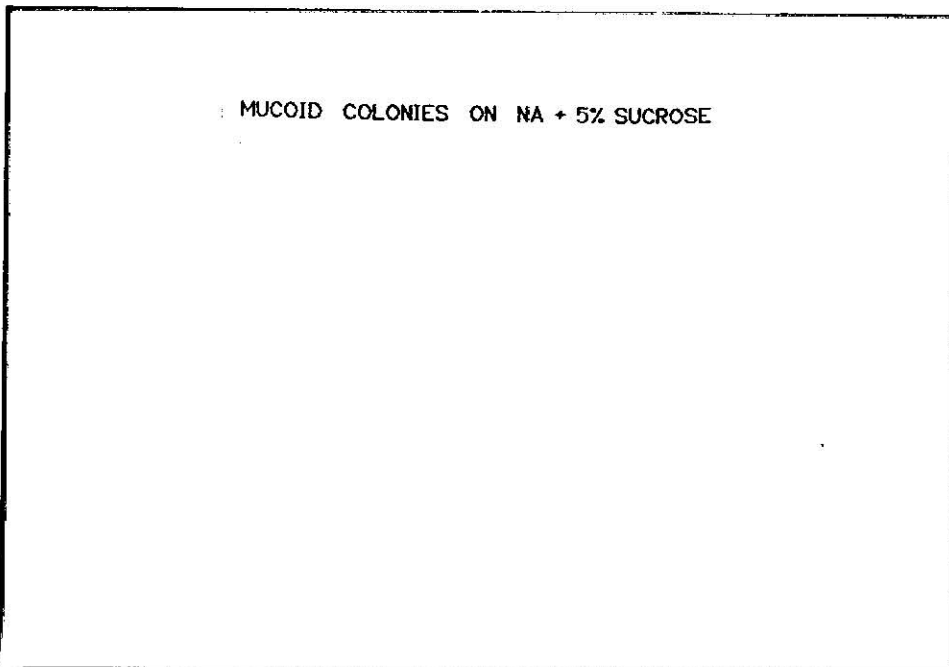
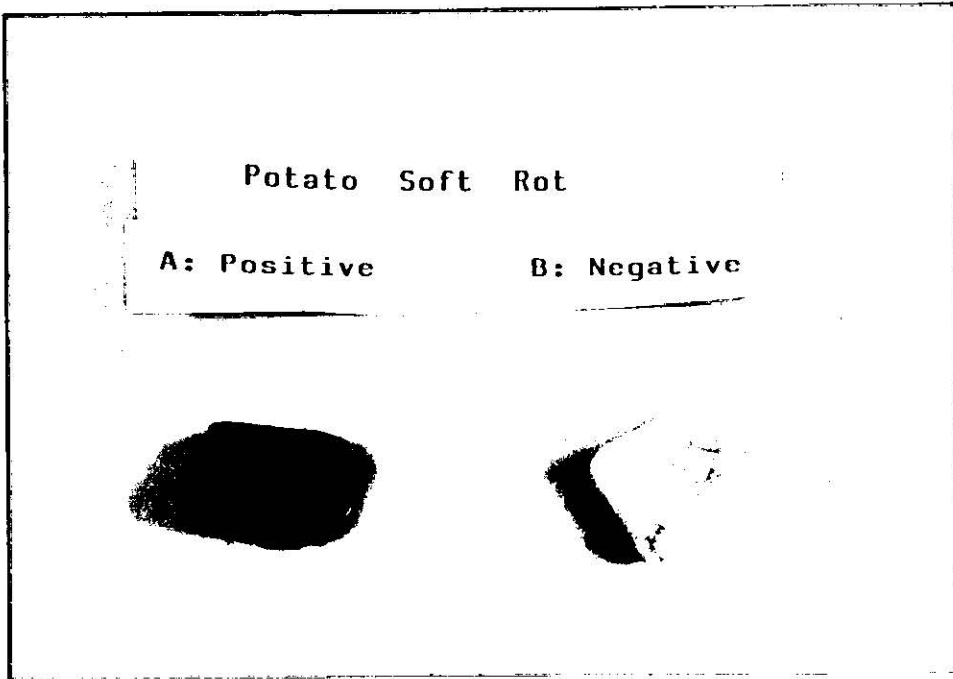
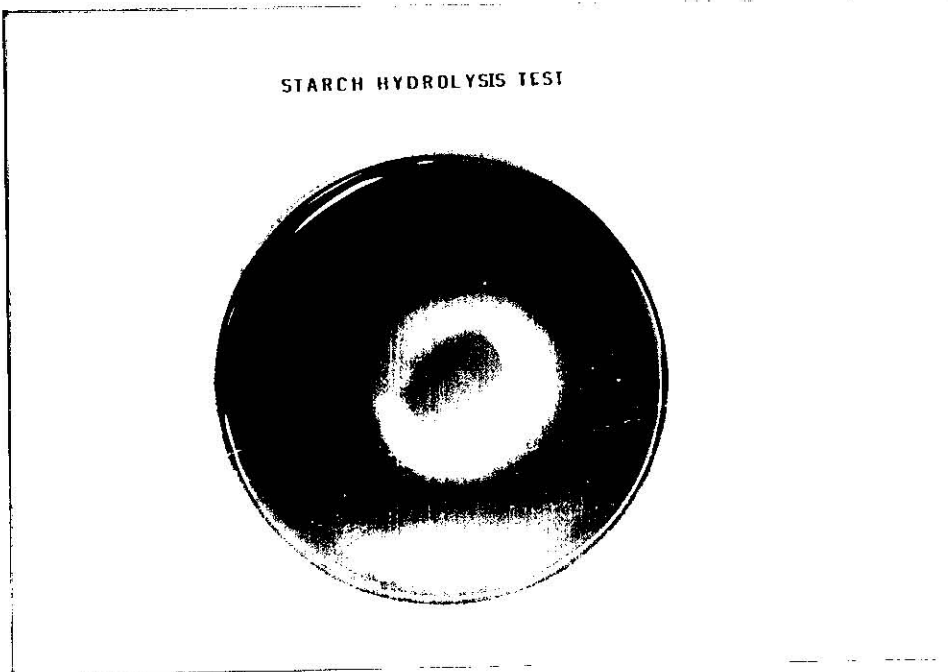


Fig. (1):

D.



E.



F.

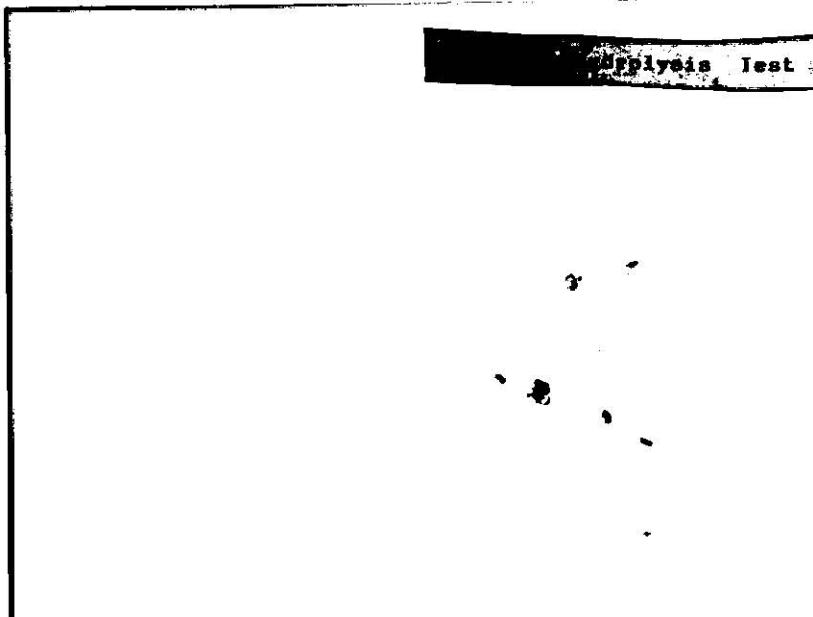


Fig. (1):

#### **4:4: Development of symptoms:**

Generally, symptoms were first observed on lower leaves. The disease started as faint yellow, water soaked spots that enlarged rapidly, and developed to necrotic spots surrounded by a lemon yellow halo. Sometimes the spot enlarged to cover the whole leaf area, (Fig. 3A). Symptoms spread to upper parts of the plant, and the infected leaves defoliated early.

Pods showed various degrees of infection ranging from just small water-soaking lesions, to elongated blotches accompanied by yellow bacterial ooze on their surface, later dried and turned greasy to brown spots. Severe infection appeared as reddish brown areas reducing the marketable value of pods (Fig. 3B).

The infected seeds showed yellow spots around the hilum region, symptoms are more clear on infected white bean seeds. Severely infected seeds were smaller in size, shrunk and shriveled appearing as yellow masses (Fig. 3C)

#### **4:5: Disease Development :**

##### **Experiment no. 1:**

Common blight developed rapidly on all tested cultivars, during the first three weeks, especially on Kentucky Wonder and Trophy. Disease incidence increased on most tested cultivars and reached a peak after 5-7 weeks. The disease incidences were : 41.3, 70.0, 31.0,

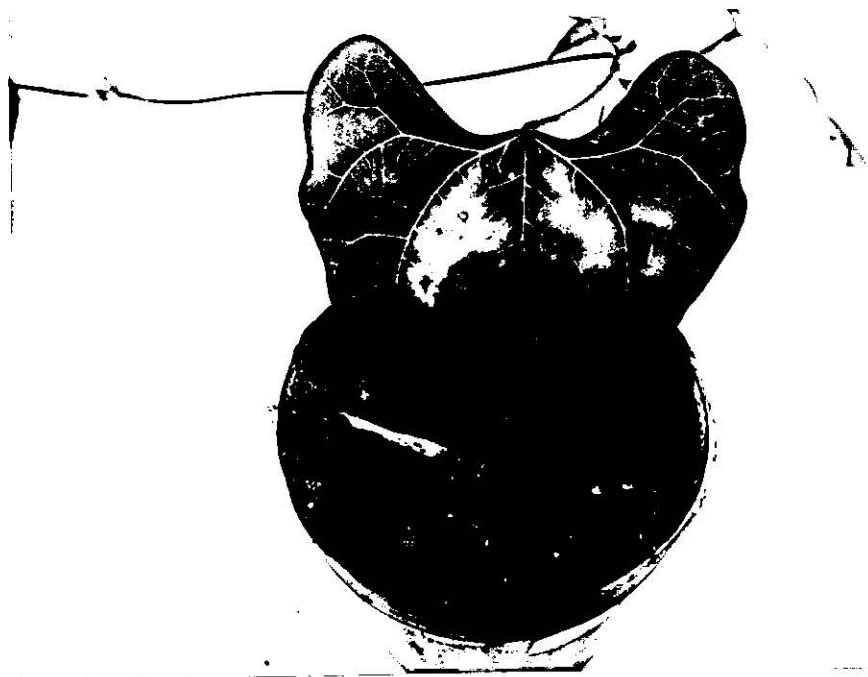
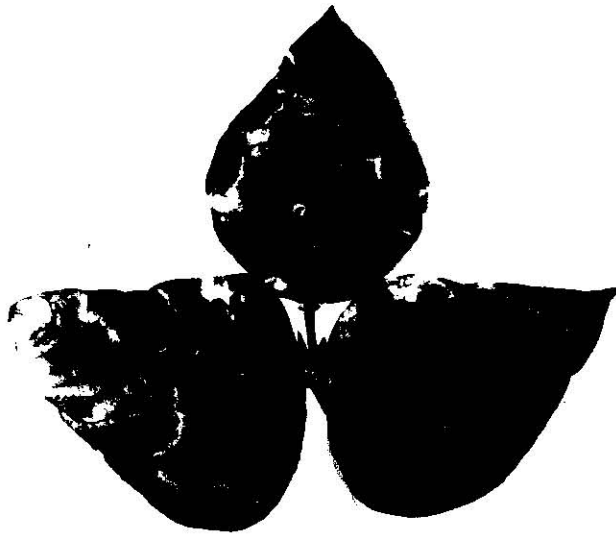
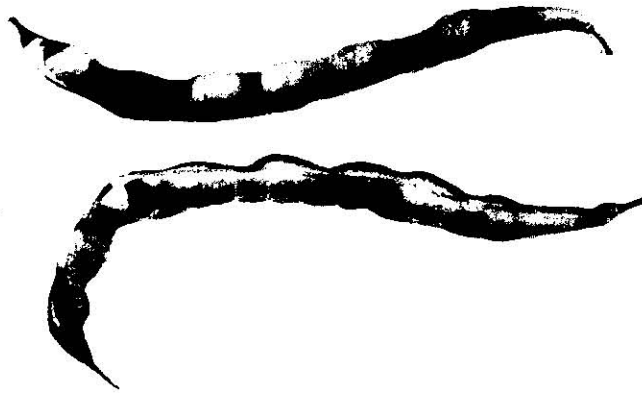


Fig.(2): Artificially Inoculated Bean Seedling Leaf Showing Positive Reaction For Xanthomonas campestris pv. phaseoli.

A



B



C

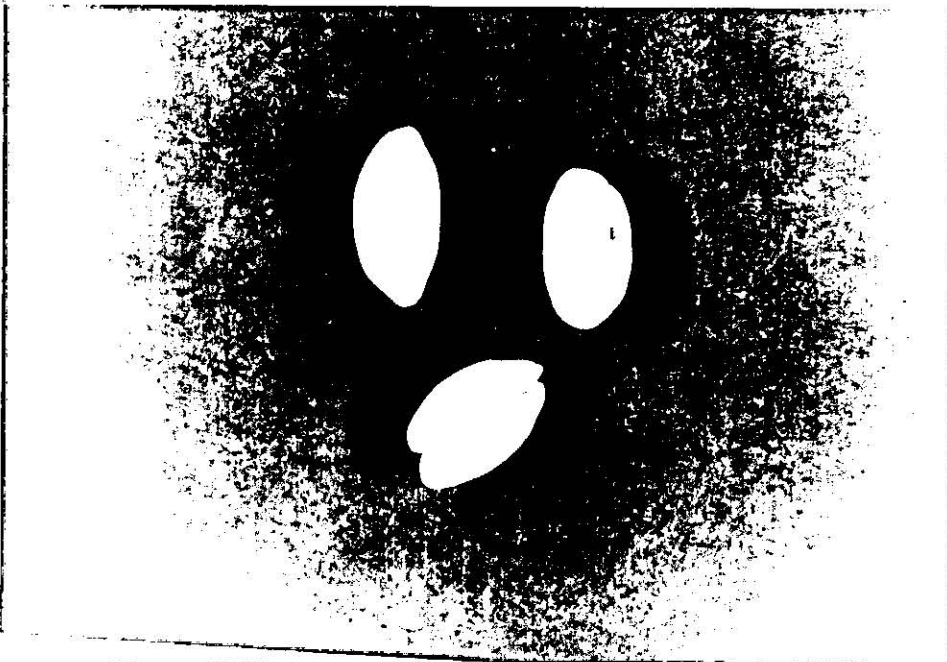


Fig. (3): Symptoms of Common Blight of Beans on:

A. Leaves B. Pods C. Seeds



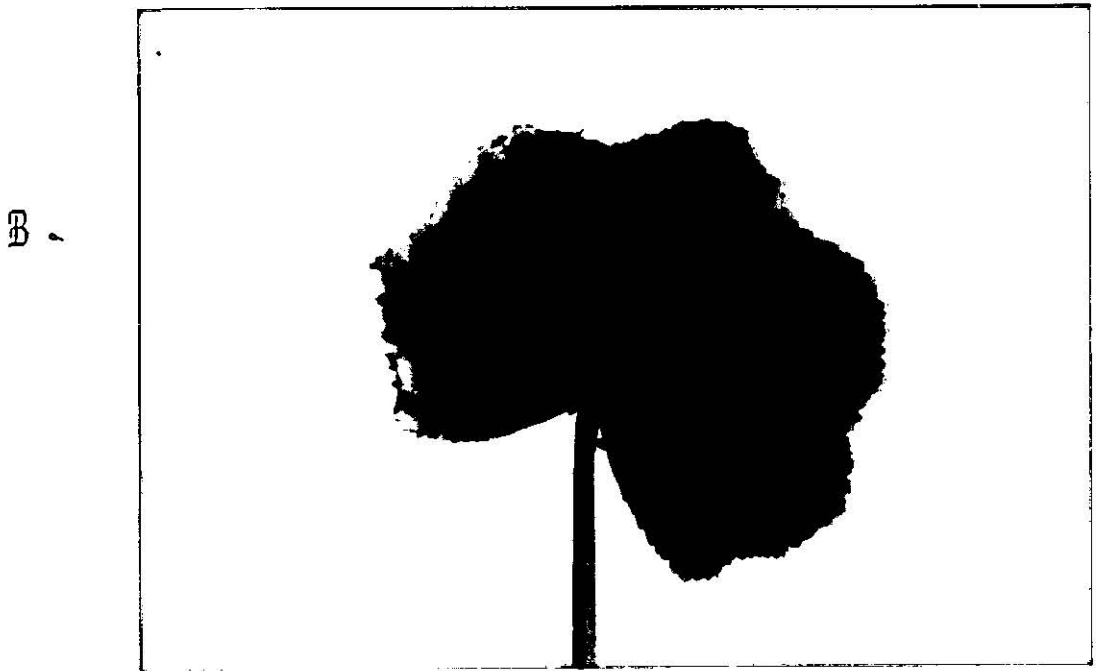


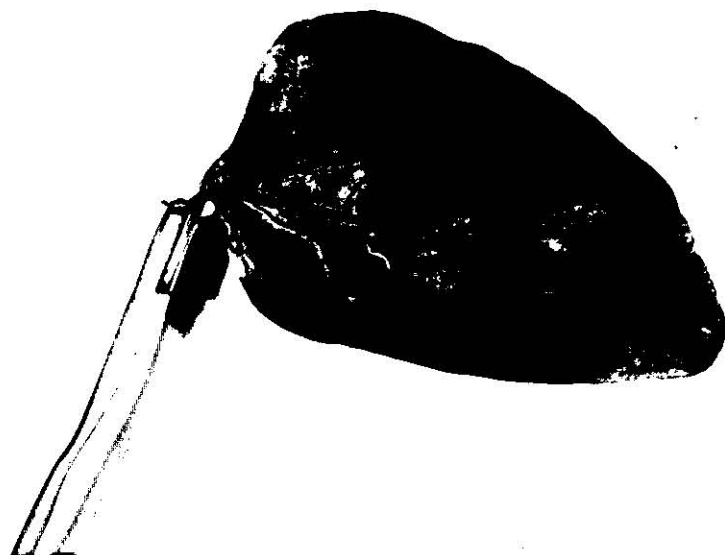
Fig. (4): Symptoms of *Xanthomonas campestris* pv.

*Phaseoli* on:

A. *Ipomoea purpurea*

B. *Malva syriaca*

C



D



Fig. (4): Symptoms of *Xanthomonas campestris* pv.

*Phaseoli* on:

C. *Vigna unguiculata*

D. *Vigna luteola*

23.5, 38.0, 33.9, 58.4, and 27.2% for Contissa, Kentucky Wonder, Lollita, Nerina RS, Strike, Tema, Trophy, and Wade, respectively. (Fig. 5).

This rapid development of the disease coincided with high temperatures of 16.0-28.0 °C (Fig. 6) and relative humidities of 36-85% (Fig. 7) during the period extended from week no.3 to week no.7

**Experiment no. 2 :**

Compared to fall lower disease incidences of common blight was recorded on all tested cultivars, with slight fluctuation until the end of the season, where incidences reached a peak at week no. 10. The recorded incidences were 45.3, 51.6, 37.6, 23.0, 33.2, 63.8, and 16.9%, for Contissa, Kentucky Wonder, Lollita, Strike, Tema, Trophy, and Wade, respectively (Fig. 8), except for Nerina RS, where incidence reached the highest value (35.1%) at week no. 8. The incidence peak coincided with temperatures of 11.6- 37.9 °C (Fig. 9), and relative humidities of 32.9- 81.4% (Fig. 10) for minimum and maximum, respectively.

The incidence of the disease on most of the tested cultivars dropped at week no. 9 (Fig. 8), without great fluctuation in temperature and relative humidity.

**Experiment no. 3 :**

Common blight developed rapidly on all tested

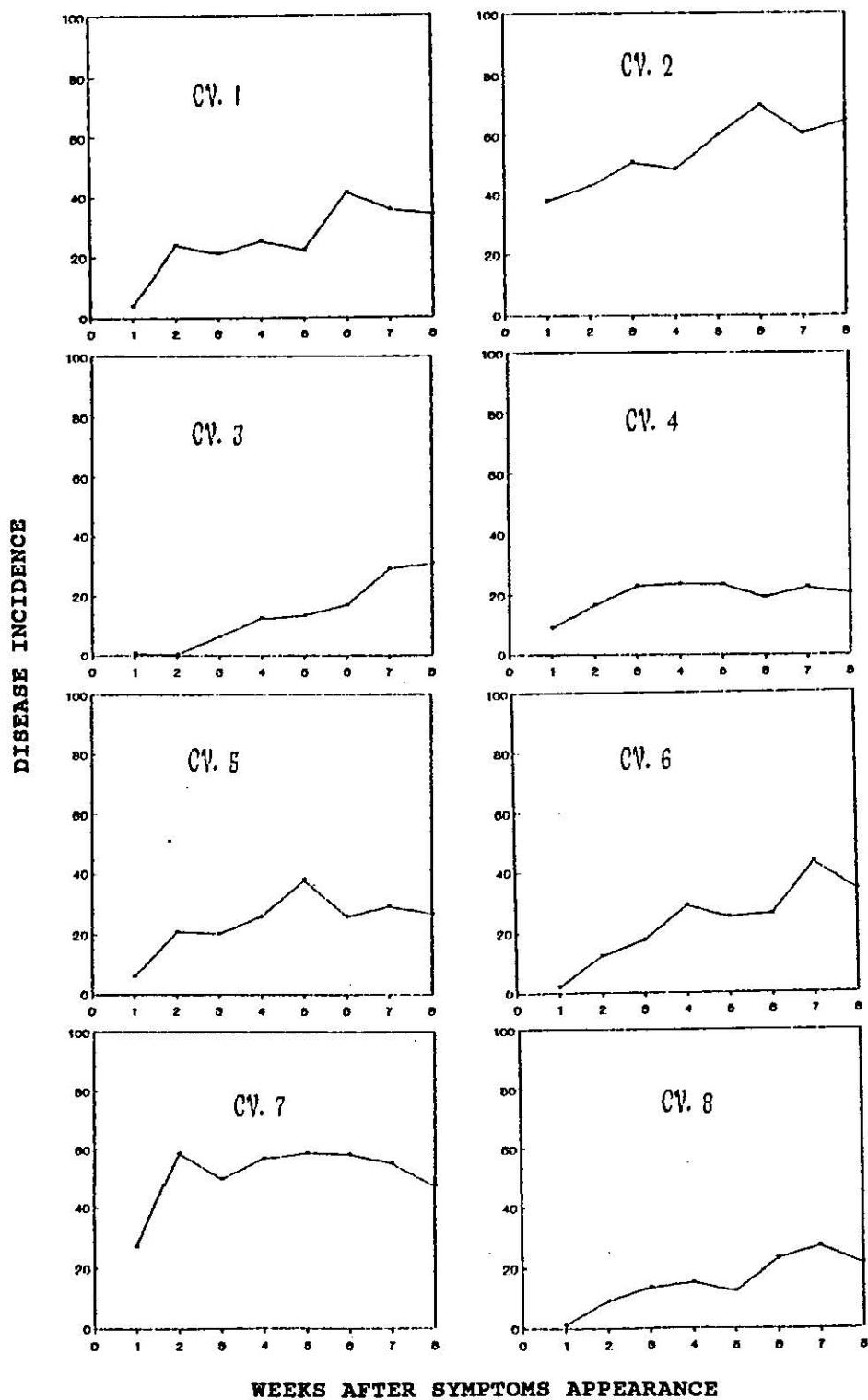


Fig. (5) : Development of common blight on tested bean cultivars in the open field during fall, 1991 (Experiment no.1).

CV.1 = Contissa      CV.2 = Kentucky Wonder      CV.3 = Lollita  
 CV.4 = Nerina RS      CV.5 = Strike      CV.6 = Tema  
 CV.7 = Trophy      CV.8 = Wade

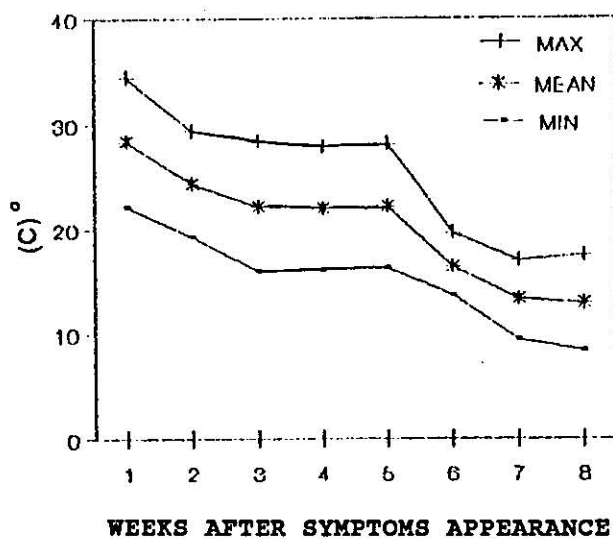


Fig. (6) : Average temperature in the open field for experiment no.1, fall, 1991.

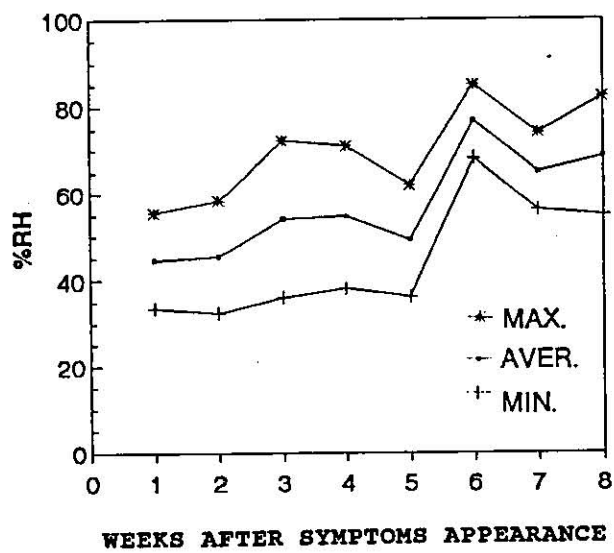


Fig. (7) : Average relative humidity in the open field for experiment no.1, fall, 1991.

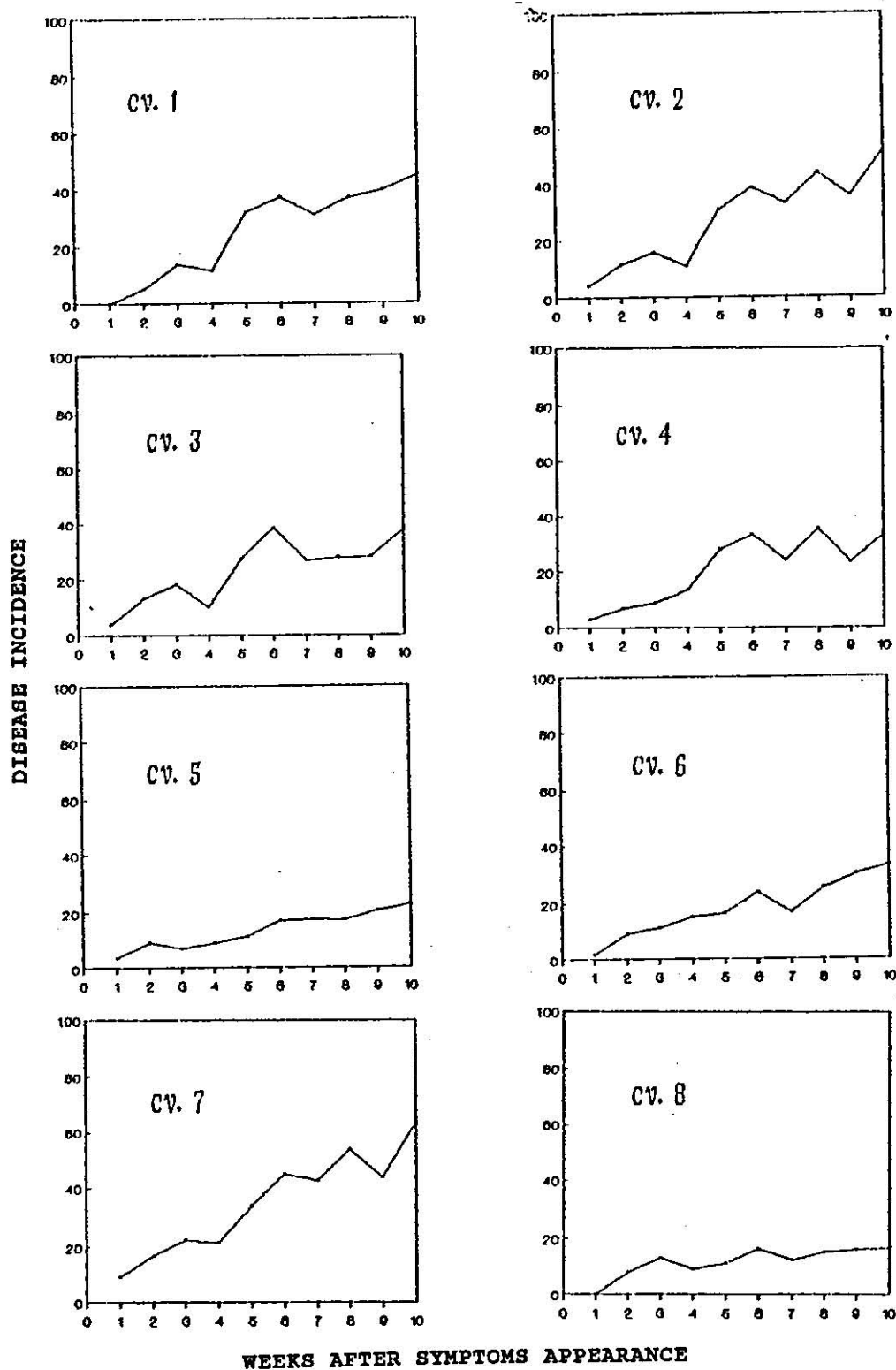


Fig. (8) : Development of common blight on tested bean cultivars inside the plastic house during winter 1991/92 (Experiment no.2).

CV.1 = Contissa      CV.2 = Kentucky Wonder      CV.3 = Lollita  
 CV.4 = Nerina RS      CV.5 = Strike      CV.6 = Tema  
 CV.7 = Trophy      CV.8 = Wade

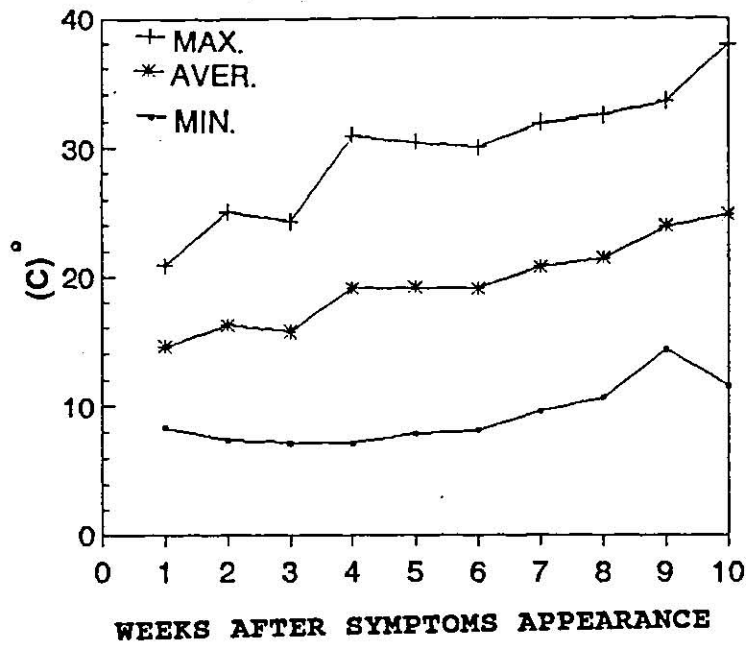


Fig. (9) : Average temperature inside the plastic house for experiment no.2, winter, 1991/92.

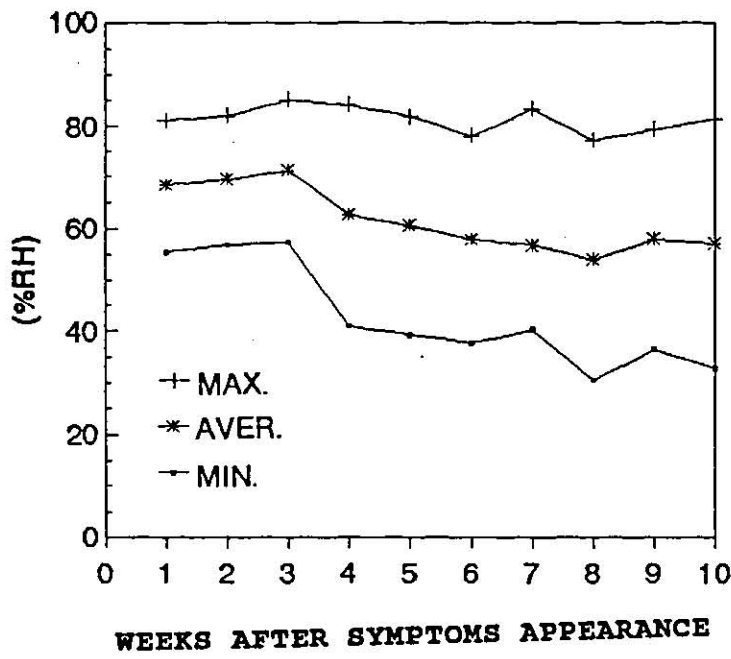


Fig. (10) : Average relative humidity inside the plastic house for experiment no.2, winter, 1991/92.

cultivars during the period extending from week no. 2 to week no. 5 (Fig. 11). This rapid development coincided with temperatures ranging from 14.9 to 33.8 °C (Fig. 12) and relative humidities of 29.3-69.1% (Fig. 13). During the following weeks the incidence fluctuated slightly until the peak was reached at week no.8, where the recorded incidences were : 86.5, 64.6, 46.3, 22.8, 26.0, 91.4 and 23.5% for Kentucky Wonder, Lollita, Nerina RS, Strike, Tema, Trophy, and Wade, respectively. While disease incidence on the cultivar Contissa reached its peak at week no. 5 (58.3%). The incidence peaks coincided with temperatures ranging from 24.0 to 39.5 °C (Fig. 12), and relative humidities of 28.9- 60.4% (Fig. 13).

#### **4:6: Response of the tested bean cultivars to common blight**

:

All the tested cultivars were found to be infected with the disease at various degrees of susceptibility in the three planting dates as follows :

##### **Experiment no. 1 :**

The tested bean cultivars could be divided according to Peterson et al. (1948) scale into two groups :

**1- Moderately resistant :** This included Lollita and Wade cultivars. The average disease incidence values were 13.7 and 15.2%, respectively. There was no significant differences between them. Also there was no significant



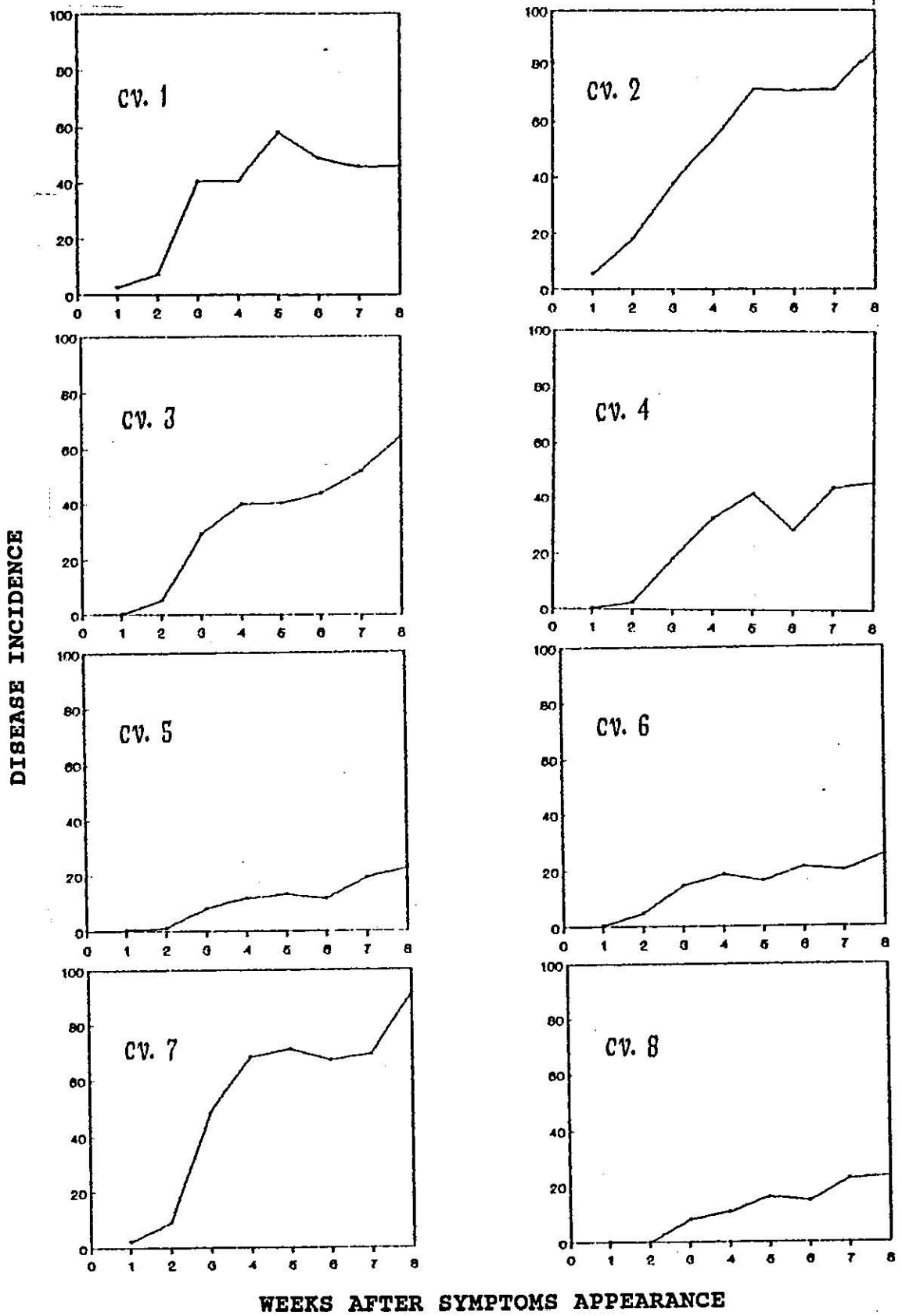


Fig. (11): Development of common blight on tested bean cultivars in the open field during spring, 1992 (Experiment no.3)

CV.1 = Contissa      CV.2 = Kentucky Wonder      CV.3 = Lollita  
 CV.4 = Nerina RS      CV.5 = Strike      CV.6 = Tema  
 CV.7 = Trophy      CV.8 = Wade

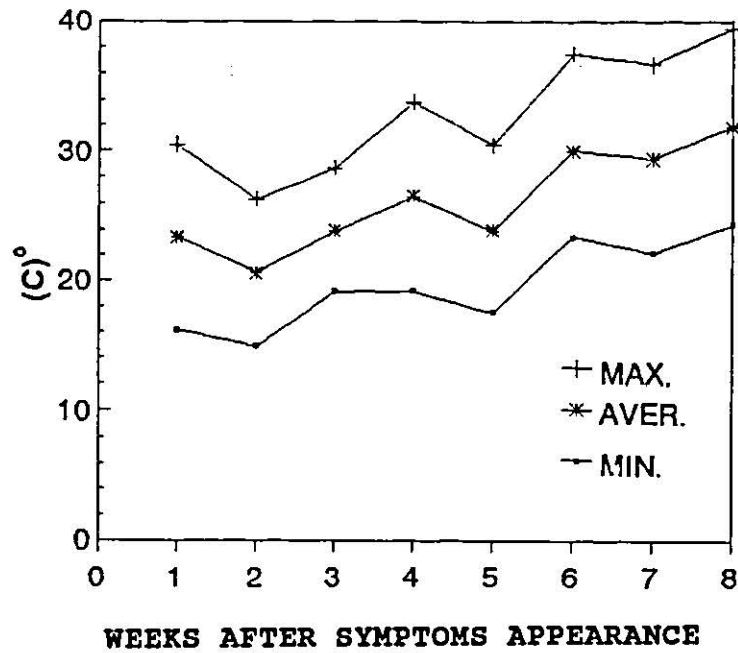


Fig. (12) : Average temperature in the open field for experiment no.3, spring, 1992.

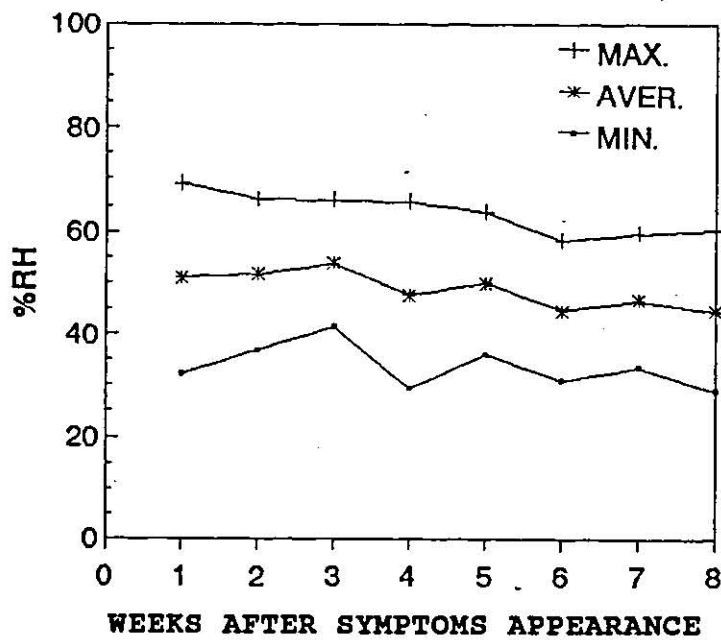


Fig. (13) : Average relative humidity in the open field for experiment no.3, spring, 1992.

differences between the cultivars Contissa, Nerina RS, Tema, and Strike. The averages of disease incidence were 24.1, 19.7, 21.8, and 24.1%, respectively. In addition, severity values for the above mentioned cultivars ranged between 0.9 and 1.8 (Table 3) .

**2- Susceptible :** This included Kentucky Wonder and Trophy cultivars with incidence values of 54.4, and 51.7%, and severity values of 3.2 and 3.4, respectively. No significant differences were detected between values of both incidence and severity.

**Experiment no. 2 :**

The tested cultivars could be separated into the following groups (Table 4) :

**1- Moderately resistant :** This included Lollita, Nerina RS, Tema, Strike, and Wade, with average incidences of 23.2, 21.0, 18.4, 13.7, 12.0%, and severity values of 2.3, 2.3, 1.8, 1.3, and 1.3, respectively. However, there was no significant difference between the first two cultivars. Also there was no significant differences between Nerina RS and the other two cultivars (Tema and Lollita), even though the later two cultivars varried significantly.

**2- Moderately susceptible :** This included Contissa, Kentucky Wonder and Trophy with average incidences of 25.6, 27.9, and 35.4%. and severity values of 2.3, 2.2, and 3.1, respectively. The first two cultivars didn't differ significantly regarding incidence and severity values. However, both differed significantly from the third

TABLE (3) : Average\* common blight incidence, severity, and the category of susceptibility of the tested bean cultivars in experiment no. 1.

Cultivar	Incidence**	Severity**	Category
Contissa	24.1 b	1.8 b	MR
Kentucky Wonder	54.5 a	3.2 a	S
Lollita	13.7 d	1.3 c	MR
Nerina RS	19.7 cd	1.4 c	MR
Strike	24.1 bc	1.2 c	MR
Tema	21.8 bc	1.5 c	MR
Trophy	51.7 a	3.4 a	S
Wade	15.2 d	0.9 d	MR
L.S.D. (p=0.05)	6.0	0.29	--

\* Average of 4 replicates and 8 times.

\*\* Figures followed by the same letter are not significantly different.

TABLE (4) : Average\* common blight incidence, severity, and the category of susceptibility of the tested bean cultivars in experiment no. 2.

Cultivar	Incidence**	Severity**	Category
Contissa	25.6 bc	2.3 b	MS
Kentucky Wonder	27.9 b	2.2 b	MS
Lollita	23.2 cd	2.3 b	MR
Nerina RS	21.0 de	2.3 b	MR
Strike	13.7 f	1.3 d	MR
Tema	18.4 e	1.8 e	MR
Trophy	35.4 a	3.1 a	MS
Wade	12.0 f	1.3 d	MR
L.S.D. (p=0.05)	2.9	0.52	--

\* Average of 4 replicates and 10 times.

\*\* Figures followed by the same letter are not significantly different.

TABLE (5): Average\* common blight incidence, severity, and the category of susceptibility of the tested bean cultivars in experiment no. 3.

Cultivar	Incidence**	Severity**	Category
Contissa	36.3 b	2.3 b	MS
Kentucky Wonder	51.9 a	3.5 a	S
Lollita	34.5 b	2.3 b	MS
Nerina RS	27.0 c	2.0 c	MS
Strike	11.1 d	0.9 e	MR
Tema	15.3 d	1.2 d	MR
Trophy	53.2 a	3.5 a	S
Wade	12.0 d	1.2 d	MR
L.S.D. (p=0.05)	4.9	0.22	--

\* Average of 4 replicates and 8 times.

\*\* Figures followed by the same letter are not significantly different.

cultivar (Trophy).

**Experiment no. 3 :**

Results in (Table 5) revealed the following categories

:

1- Moderately resistant : This included Strike, Tema, and Wade, with average incidence of 11.1, 15.3 and 12.1%, and severity values of 0.9, 1.2, and 1.2, respectively. There was no significant differences between the three cultivars regarding the incidence of the disease; however, Strike cultivar differed significantly from the other two cultivars regarding severity.

2- Moderately susceptible : This included Contissa, Lollita, and Nerina RS, with average incidence of 36.3, 34.5, and 27.0% , and severity values of 2.3, 2.3, and 2.0, respectively. No significant differences occurred between the first two cultivars, however, Nerina RS differed significantly from them.

3- Susceptible : This category included Kentucky Wonder and Trophy with 51.9 and 53.2% incidences, and 3.5 and 3.5 severity values, respectively. There was no significant difference between both cultivars.

**4:7: Sources of inoculum :**

**4:7:1: Seeds :**

Pathogenic strains of Xcp were recovered from seed soaks of all tested bean cultivars, when the seed soak was

streaked on NA medium plate (Table 6). On the other hand bean seedlings infiltrated with seed soaks of all tested cultivars developed symptoms similar to those induced on bean seedlings inoculated with a suspension of the reference culture of Xcp (ATCC 9563). While none of the seedlings inoculated with the enrichment medium, or sterile (PBS) showed any signs of the disease. The pathogen was reisolated from bean seedlings inoculated with the seed soaks of all tested cultivars (Table 6).

#### **4:7:2: Plant debris :**

Pathogenic strains of Xcp were recovered from bean debris collected at monthly intervals from the field during the period extended from June until October; at which the Jordan Valley is free from cultivated bean (Table 7). However, the number of Xcp colony forming units per 1 g (CFU/g) of bean debris isolated on MXP medium, decreased by time (Fig. 14). All seedlings inoculated with debris soak showed symptoms similar to those induced on seedlings infiltrated with suspension of the reference culture of Xcp (ATCC 9563) throughout the whole period and the pathogen was reisolated from bean seedlings inoculated with soak of all tested cultivars. No symptoms developed on seedling inoculated with (PBS).

#### **4:7:3: Soil :**

No pathogenic Xcp strain was recovered from any of the tested soil samples collected at monthly intervals

الصفحة غير موجودة من أصل المصدر



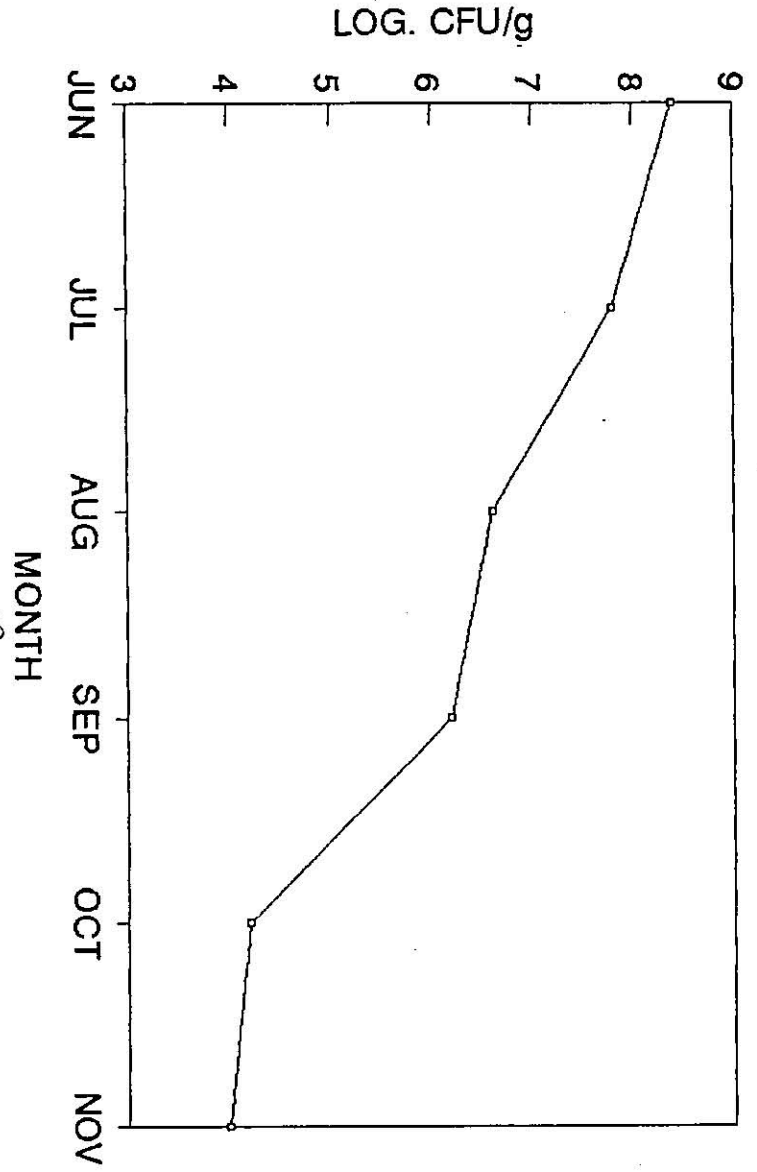


Fig (14) : Log. Colony Forming Units (CFU) of Xcp per 1 gram bean debris collected at monthly intervals during the period June-November, 1992.

TABLE (8) : Detection of Xcp from soil samples collected from different areas in the Jordan Valley, at monthly intervals.

Month	Colony	Northern Shouneh	Wadi El Yabis	Deir-Alla	Almasri Triangle	Karameh	Southern Shouneh	Wadi Shoaib
May	YC 1	+	+	+	+	+	+	+
	Xcp 2	-	-	-	-	-	-	-
June	YC	+	+	+	+	+	+	+
	Xcp	-	-	-	-	-	-	-
July	YC	+	+	-	+	-	+	+
	Xcp	-	-	-	-	-	-	-
Aug.	YC	-	-	-	-	-	-	-
	Xcp	-	-	-	-	-	-	-
Sep.	YC	-	+	-	-	-	-	-
	Xcp	-	-	-	-	-	-	-

1 Yellow colonies on NA medium.

2 *Xanthomonas campestris* pv. *phaseoli*.

respectively.

Oxytetracycline and Diathane M-45 were less effective, with incidences of 16.6 and 16.9% and severity values of 1.0 and 1.2, respectively.

All tested compounds resulted in significantly higher average yield than the check. Tri-Miltox remained the most significantly effective compound.

TABLE (9) : Average incidence \*, severity\*, and yield of the four replicates of the different treatments.

Treatment	Incidence	Severity	Marketable Yield (Kg)	
			Average	Total
Tri-Miltox	7.2 c	0.68 c	4.6 a	18.4
Cuprosan	9.4 c	0.80 bc	1.9 b	7.6
Ditiver	10.2 c	0.82 bc	2.0 b	8.0
Diathane-M-45	16.9 b	1.15 bc	2.5 b	10.0
Oxytetracycline	16.6 b	1.02 bc	2.6 b	10.4
Check	53.5 a	3.24 a	0.7 c	2.8
L.S.D. (p=0.05)	3.34	0.29	0.9	---

\* Average of 8 times.

\*\* Figures followed by the same letter are not significantly different.

The reactions of the different obtained bacterial isolates collected from the different growing areas in the Jordan Valley to the different tests carried out in this study were similar and identical to the reactions of the reference culture of Xcp (ATCC 9563). This means that the causal agent of common blight of bean in Jordan is [*Xanthomonas campestris* pv. *phaseoli* (Smith) Dye.]

Field inspection revealed that the disease occurred in all bean growing areas in the Jordan Valley.

The recorded disease incidence values were higher under open field conditions, than under the plastic-house. This may be attributed to : the exceptional low temperatures recorded under the plastichouse during the growing season of spring 1991/92, plant growth of the tested cultivars was more vigorous than in the open field. Similar results were obtained by Andrus (1948); he found that susceptible reaction of bean cultivars to common blight was due to the poor growth of the host. Also, plants grown under the plastichouses are less exposed to wind and rain, which were known as good means for common blight spread (Weller and Saettler, 1980 A). The high disease incidences in the first experiment during the sixth and seventh weeks were coincided with high rainfall which could help in the dissemination of pathogen and favour disease development. However, the decline in disease incidences without much fluctuation in

temperatures and relative humidity may be related to the appearance of new vegetation on the different cultivars.

Common blight developed at temperature ranges of : 8.4-34.5, 7.1-37.9, and 14.9-39.5 °C, and relative humidity ranges of 33.6 -85.1, 30.6-85.1, and 29.3-69.1% for experiments no.1, no.2, and no.3, respectively. Rapid development of common blight coincided with average temperatures of : 16.0-34.5, 11.6-37.9, and 14.9-33.8 °C and average relative humidities of 32.4-58.4, 32.9-81.4, and 29.3- 69.1%, for the three experiments, respectively. This suggests that average temperature of about 24 °C and relative humidity of 50% and above could be considered as optimal conditions for the disease development. These results are in general agreement with the findings of Hayward and Waterson (1965) and Venette (1978); they found that Xcp is a warm pathogen developing optimally at a temperature of 28 °C accompanied with high rainfall.

Disease development in the three experiments followed the same trend on all tested cultivars , with some deviations, which could be attributed to the differences in environmental conditions (Webster et al., 1983).

All tested cultivars were susceptible with various degrees, and non of them was resistant or immune to the disease. However high levels of resistance is not reported in beans against common blight (Zapata, 1985). Kentucky Wonder and Trophy were the most susceptible cultivars , while Tema, Strike, and Wade were found to be more tolerant ones than the other tested cultivars.

Results of this study indicated that seeds play an

الصفحة غير موجودة من أصل المصدر

## 6: CONCLUSIONS

- 1- The results of biochemical and physiological tests revealed that the causal agent of common blight of beans in Jordan Valley is *Xanthomonas campestris* pv. *phaseoli*.
- 2- Common blight occurred in all bean growing areas in the Jordan Valley.
- 3- The disease was found to develop at temperatures ranging from 7.1 to 39.5 °C and relative humidities above 30% . Temperature means of 24 °C and relative humidity above 50% were found to be optimal for disease development.
- 4- Non of the tested bean cultivars was found to be highly resistant to common blight. However, Strike, Tema, and Wade were found to be more tolerant than the other tested bean cultivars.
- 5- Infected bean seeds and bean debris constitute the main source of primary inoculum.
- 6- The following plants could be considered as new hosts for Xcp:

*Ipomoea purpurea*      *Malva syriaca*      *Pisum sativum*  
*Vigna luteola*      *Vigna unguiculata*

- 7- Spraying with fixed copper compounds suppressed common blight and resulted in higher marketable yield.

Tri-Milttox      was the most effective compound.

## REFERENCES

أبو بلان، حفطي، وحامد خليف . تقدير نسبة وشدة الإصابة بالامراض الفطرية والبكتيرية على اصناف الفاصولياء المزروعة بداخل الدفيئات في غور الاردن . مجلة مؤتة للبحوث ولادراسات . (مقبول للنشر)

Aggour, A. R., Dermot, P., Coyne, D. P. , and Vidavar, A. K. 1989. Comparison of leaf and pod disease reaction of bean (*Phaseolus vulgaris*) inoculated by different methods with strains of [*Xanthomonas campestris* pv. *phaseoli*] (Smith) Dye. *Euphytica*. 43:143-152.

Andrus, C. F. 1948. A method of testing beans for resistance to bacterial blights. *Phytopathology* 38: 757-759 (Abst.).

Angeles-Ramos, R., Vidavar, A. K., and Flynn, P. 1991. Characterization of epiphytic [*Xanthomonas campestris* pv. *phaseoli*] and pectolytic *Xanthomonads* recovered from symptomless weeds in the Dominican Republic. *Phytopathology*. 81: 677-681.

Araund-Santana, E., Pena-Motos, E. Coyne, D. P., and Vidavar, A. K. 1991. Longevity of [*Xanthomonas campestris* pv. *phaseoli*] in naturally infected dry bean (*Phaseolus vulgaris*) debris. *Plant Disease*.75: 952-953.

Arias, A. D. O. 1989. Correlation between foliar resistance and seed infection in bean varieties inoculated with [*Xanthomonas phaseoli*]. Pirscaba. Brazil. 105 pp. (Abst.).

Cafati, C. P., and Saettler, A. W. 1980 A. Effect of host on multiplication and distribution of common blight bacteria. *Phytopathology* 70: 675-679.



**Cafati, C. P., and Saettler, A. W. 1980 B.** Role of nonhost species as alternate inoculum sources of [*Xanthomonas phaseoli*] . Plant Disease 64: 194-196.

**Cafati, C. P., and Saettler, A. W., 1980 C.** Transmission of [*Xanthomonas phaseoli*] in seeds of resistant and susceptible (*Phaseolus vulgaris*) genotypes. Phytopathology 70: 638-640.

**Claflin, L. E., Vidavar, A. K., and Sasser, M. 1987.** MXP, a semi-selective medium for [*Xanthomonas campestris* pv. *phaseoli*]. Phytopathology 77: 730-734.

**Copeland, L. O., Adams, M. W., and Bell, D. C. 1975.** An improved seed program for maintaining disease-free seed of field beans (*Phaseolus vulgaris*). Seed Science Technology 3: 719-724.

**Departement of General Statistics. 1991.** Annual Report. Amman, Jordan.

**Dermot, B. P. Coyne, D. P., and Schuster, M. L. 1973.** *Phaseolus* germplasm tolerant to common blight bacterium [*Xanthomonas phaseoli*] . Plant Disease Reporter 57 (2): 11-114.

**Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R., and Phillips, G. B. 1981.** Manual of Methods for General Bacteriology.

**Gilbertson. R. L., Rand, R. E., Carlson, E., and Hagendoron, D. J. 1988.** The use of dry-leaf inoculum for establishment of common bacterial blight of beans. Plant Disease 72: 385-389.

- Gilbertson, R. L., Rand, R. E., and Hagendorn, D. J. 1990. Survival of [*Xanthomonas campestris* pv. *phaseoli*] and pectolytic strains of [*X. campestris*] in bean debris. *Plant Disease* 74: 322-327.
- Graham, J. H. 1953. Overwintering of three bacterial pathogens of soybean. *Phytopathology* 43: 189-192. (Abst.)
- Hayward, A. C., and Waterson, J. M. 1965. [*Xanthomonas phaseoli*] No. 48. In : Description of Plant Pathogenic Fungi and Bacteria. Commonwealth Mycological Institution, England.
- Horsfall, J. G. and Hueberger, J. W. 1942. Measuring magnitude of a defoliation disease of tomatoes. *Phytopathology* 32: 226-232.
- Khlaif, H. M. 1986. Serological Detection of Bacterial Pathogens from Bean. Ph. D. Thesis. Fargo North Dakota 145 pp.
- Krieg, N. R., and Holt, J. G. 1984. *Bergey's Manual of Systemic Bacteriology*. Volume 1. Williams and Wilkins. London. 963 pp.
- Lahman, L. K., and Schaad, N. W. 1985. Evaluation of the dome test as a reliable assay for seedborne bacterial blight pathogens of beans. *Plant Disease* 69: 680-683.
- Leben, C. 1965. Epiphytic microorganisms in relation to Plant Disease. *Annual Review of Phytopathology* 3: 209-230.
- Lelliot, R. A., and Stead, D. E. 1987. *Methods for Diagnosis of Bacterial Diseases of Plants*. Blackwell

- Schaad, N. W.** 1988 Laboratory Guide of Plant Pathogenic Bacteria. 2nd Edition. APS Press. 152 pp.
- Schuster, M. L.** 1955. A method for testing resistance of beans to bacterial blights. *Phytopathology* 45: 519-520.
- Schuster, M. L., and Coyne, D. P.** 1971. New virulent strains of [*Xanthomonas phaseoli*]. *Plant Disease Reporter* 55: 505-506.
- Schuster, M. L., and Coyne, D. P.** 1974. Survival mechanisms of phytopathogenic bacteria. *Annual Review of Phytopathology* 12: 199-221.
- Schuster, M. L. Coyne, D. P., Nuland, D. S., and Smith C. C.** 1974. Transmission of [*Xanthomonas phaseoli*] and other bacterial species or varieties in seeds of tolerant bean (*Phaseolus vulgaris*) cultivars. *Plant Disease Reporter* 63: 955-959.
- Sharen, A. L.** 1959. Comparative population trends of [*Xanthomonas phaseoli*] in susceptible field tolerant, and resistant hosts. *Phytopathology* 49: 425-428.
- Stanek, M., and Lasik, J.** 1965. The occurrence of microorganisms parasitizing on the over-ground parts of plants in the rhizosphere. Maeura, H, and Vancura, V., editions. Pages 300-307 in : *Plant-Microbe Relationships*. Published by Progue Publ. House.
- Venette, J. R.** 1978. Disease and bean certification. *North Dakota Seed Journal*. December p. 3.
- Venette, J. R., and Lamey, H. A.** 1978. Dry edible bean diseases. *North Dakota Corporation Extension Service*. No. 576. 8pp.

Webster, D. M. Atkin, J. D. and Cross, J. E. 1983 A. Bacterial blights of snap beans and their control. Plant disease 67: 935-940.

Webster, D. M. Tebble, S. R. and Glavez, G. 1983 B. Expression of resistance to [*Xanthomonas campestris phaseoli*] in [*Phaseolus vulgaris*] under tropical conditions. Plant Disease 67: 334-340.

Weller, D. M., and Saettler, A. W. 1980 A. Colonization and distribution of [*Xanthomonas phaseoli*] and [*Xanthomonas phaseoli* var. *fuscans*] in field grown navy beans. Phytopathology. 70 : 500-506.

Weller, D. M., and Saettler, A. W. 1980 B. Evaluation of seed-borne [*Xanthomonas phaseoli*] and [*Xanthomonas Phaseoli* var. *fuscans*] primary inocula in bean blights. Phytopathology. 70 : 148-152.

Yoshi, K. Galver, G. E., and Alvarez, A. 1978. Screening bean germplasm for tolerance to common blight caused by [*Xanthomonas phaseoli*] and importance of pathogenic variation to varietal improvement. Plant Disease Reporter 62: 343-347.

Zapata, M. Freytag, G. F. and Wilkinson R. E. 1985. Evaluation for bacterial blight resistance in beans. Phytopathology 75: 1032-1039.

Appendex (1) : Weekly means of Incidence of common blight for experiment no. 1.

Cultivar	Week1	Week2	Week3	Week4	Week 5	Week 6	Week 7	Week 8	Mean
Contissa	3.8 cd	23.6 c	20.4 b	24.9 bcd	22.0 c	41.3 c	35.4 b	33.8 c	24.1 b
Kentucky Wonder	37.7 a	43.0 b	51.0 a	48.9 a	59.8 a	70.0 a	60.6 a	64.7 a	54.5 a
Lollita	0.5 d	0.1 e	6.3 d	12.7 d	13.5 c	17.0 d	29.0 bc	31.0 c	13.7 d
Nerina RS	9.0 c	16.6 cd	22.9 b	23.6 bcd	23.5 c	19.0 d	22.5 c	20.4 d	19.7 cd
Strike	5.9 cd	20.8 cd	20.4 bc	26.1 bc	38.0 b	25.8 d	29.2 bc	26.8 cd	24.1 bc
Tema	1.7 cd	12.3 cd	17.6 bc	28.7 b	24.9 bc	26.1 d	24.8 c	33.9 c	21.8 bc
Trophy	27.2 b	58.6 a	49.9 a	57.1 a	58.7 a	58.4 b	55.3 a	47.8 a	51.7 a
Wade	0.9 d	8.9 de	13.5 c	15.0 cd	12.0 c	23.0 d	27.2 bc	21.2 d	15.2 d
L.S.D. (p=0.05)	7.4	14.2	7.2	13.3	14.0	9.2	9.7	7.3	7.3

Appendex (2) : Weekly means of severity of common blight for experimen no. 1.

	Week1	Week2	Week3	Week4	Week 5	Week 6	Week 7	Week 8	Mean
Cultivar									
Contissa	0.2 b	0.6 c	0.2 c	2.0 b	3.3 b	2.7 b	2.3 c	2.0 c	1.8 b
Kentucky	1.3 a	2.4 b	3.0 b	4.0 a	4.6 a	4.0 a	3.3 b	2.9 b	3.2 a
Wonder									
Lollita	0.1 b	0.0 e	0.0 e	1.6 bc	2.7 c	2.1 cd	1.8 d	1.7 cd	1.3 c
Nerina RS	0.3 b	0.3 cde	0.8 d	1.2 cd	2.9 bc	2.4 bc	1.8 cd	1.6 cde	1.4 c
Strike	0.2 b	0.5 cd	0.6 d	1.2 cd	2.4 d	2.0 cde	1.5 d	1.5 de	1.2 c
Tema	0.2 b	0.7 c	0.9 cd	1.9 b	2.7 c	1.9 de	1.8 d	1.7 cde	1.5 c
Trophy	1.3 a	3.0 a	4.1 a	4.0 a	4.3 a	4.4 a	3.8 a	3.4 a	3.4 a
Wade	0.1 b	0.1 de	0.2 e	0.6 d	2.0 cd	1.5 e	1.4 d	1.2 e	0.9 d
L.S.D. (p=0.05)	0.4	0.5	0.4	0.6	0.6				

Appendex (5) : Weekly means of incidence of common blight for experiment no. 3.

	Week1	Week2	Week3	Week4	Week 5	Week 6	Week 7	Week 8	Mean
Cultivar									
Contissa	2.8 b	7.5 bc	40.8 ab	40.9 c	58.3 a	48.7 b	45.5 b	45.7 c	36.3 b
Kentucky Wonder	5.3 a	18.2 a	37.9 bc	53.5 b	71.2 a	70.9 a	71.5 a	86.5 a	51.9 a
Lollita	0.4 c	5.3 cd	29.0 cd	40.1 c	40.5 b	44.0 b	52.0 b	64.6 b	34.5 b
Nerina RS	0.5 c	2.6 de	18.5 de	32.9 c	42.1 b	28.7 c	44.5 b	46.3 c	27.0 c
Strike	0.6 c	1.3 e	8.0 e	11.8 d	13.1 c	11.7 e	19.2 c	22.8 d	11.1 d
Tema	0.6 c	4.8 cd	14.6 e	18.6 d	16.5 c	21.6 cd	20.1 c	26.0 d	15.3 d
Trophy	2.4 b	9.8 b	48.6 a	68.6 a	71.4 a	63.6 a	69.7 a	91.4 a	53.2 a
Wade	0.3 c	0.0 e	8.3 e	11.0 d	16.2 c	14.7 de	22.7 c	23.5 d	12.1 d
L.S.D. (p=0.05)	1.5	3.0	10.8	11.1	13.4	8.7	12.8	11.0	4.9

Appendex (6) : Weekly means of severity of common blight for experiment no. 3.

	Week1	Week2	Week3	Week4	Week 5	Week 6	Week 7	Week 8	Mean
Cultivar									
Contissa	0.2 a	2.1 a	3.1 b	2.2 cd	3.0 c	1.9 c	2.8 bc	3.0 bc	2.3 b
Kentucky	0.3 a	2.2 a	2.8 bc	3.9 a	4.3 a	4.7 a	4.9 a	4.9 a	3.5 a
Wonder									
Lolitta	0.2 a	1.4 b	2.5 bc	2.7 bc	2.3 d	2.8 b	3.3 b	3.4 b	2.3 b
Nerina RS	0.1 a	1.1 bc	2.3 c	3.2 b	1.6 e	2.4 b	2.8 c	2.8 c	2.0 c
Strike	0.1 a	0.5 c	1.2 d	1.2 e	1.1 f	0.8 e	1.0 e	1.0 e	0.9 e
Tema	0.1 a	1.1 bc	1.5 d	1.6 de	1.4 ef	1.2 de	1.3 de	1.4 de	1.2 d
Trophy	0.3 a	2.4 a	4.1 a	4.1 a	3.6 b	4.3 a	4.6 a	4.7 a	3.5 a
Wade	0.1 a	1.0 bc	1.1 d	1.2 e	1.3 ef	1.4 cd	1.6 d	1.8 d	1.2 d
L.S.D. (p=0.05)	0.3	0.6	0.5	0.7	0.5	0.5	0.5	0.5	0.2



Appendex (3) : Weekly means of incidence of common blight for experiment no. 2.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Cultivar									
Contissa	0.0 c	5.8 d	14.1 bc	12.0 bcd	32.4 a	37.6 b	31.7 bc	37.5 bc	40.0 a
Kentucky	4.5 b	11.9 bc	16.1 bc	11.5 cd	31.4 a	38.9 ab	33.5 b	44.0 b	36.1 ab
Wonder									
Lollita	4.3 b	13.6 ab	18.3 ab	10.6 cd	27.2 a	38.6 ab	26.5 bc	27.7 de	27.9 bcd
Nerina RS	3.2 b	7.5 cd	9.4 de	13.9 bc	27.7 a	33.0 b	23.8 cd	35.1 cd	23.3 cde
Strike	3.7 b	9.3 bcd	7.2 e	9.2 d	11.6 d	17.3 c	17.7 de	17.6 fg	20.6 de
Tema	1.8 bc	9.3 bcd	11.7 cde	15.4 b	16.8 b	23.8 c	17.1 de	25.3 ef	30.0 bc
Trophy	9.1 a	17.2 a	22.7 a	21.8 a	34.1 a	45.0 a	42.7 a	53.9 a	43.7 a
Wade	0.0 c	8.1 cd	13.3 cd	9.1 d	11.4 b	16.7 c	12.4 e	15.5 g	16.5 e
L.S.D. (p=0.05)	3.0	4.8	4.6	3.4	7.2	7.4	8.7	8.6	9.3

Week 10	Mean
45.3 bc	25.6 bc
51.6 b	27.9 b
37.6 cd	23.2 cd
32.9 d	21.0 de
23.0 e	13.7 f
33.2 d	18.4 e
63.8 a	35.4 a
16.9 e	12.0 f
8.6	2.9

Appendix (4) : Weekly means of severity of common blight for experiment no. 2.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Cultivar	0.3 d	1.4 e	2.4 ab	3.2 a	2.5 ab	2.4 ab	2.8 b	2.6 b	3.1 ab
Contissa	1.2 c	2.2 bcd	2.0 bc	2.6 a	2.1 c	2.5 bc	2.1 c	1.9 bc	2.8 bc
Kentucky Wonder	1.8 ab	2.8 ab	2.5 ab	3.0 a	2.8 a	2.0 bc	1.9 cd	1.6 c	2.4 cd
Lolita	1.9 a	3.0 a	2.5 ab	2.9 a	2.2 bc	2.2 b	2.7 b	1.9 c	2.0 d
Nerina RS	1.3 c	1.6 de	1.6 c	1.4 b	1.2 d	1.0 de	1.3 e	1.4 c	1.2 f
Strike	1.5 abc	2.4 abc	2.0 bc	1.8 b	1.3 d	1.5 cd	1.5 de	1.9 bc	1.9 de
Tema	1.4 bc	2.4 abc	2.7 a	2.6 a	2.8 a	2.9 a	4.0 a	3.7 a	3.5 a
Trophy	1.0 c	2.0 cde	1.7 c	1.2 b	1.1 d	0.8 e	1.3 e	1.5 c	1.3 ef
Wade	0.6	0.7	0.6	0.7	0.4	0.7	0.5	0.7	0.7
L.S.D. (p=0.05)									

Week 10	Mean
2.7 bc	2.3 b
3.3 b	2.2 b
2.6 cd	2.3 b
1.5 ef	2.3 b
1.3 f	1.3 d
2.1 de	1.8 c
4.4 a	3.1 a
1.5 ef	1.3 d
0.5	0.2

## الملخص

## لفحة الفاصولياء الشائعة في وادي الأردن

تم جمع عينات من نباتات فاصولياء بدت عليها أعراض يشتهب بأنها أعراض مرض اللفحة العادية البكتيرية من مناطق الزراعة المختلفة في غور الأردن وعلى مدار الموسم الزراعي ١٩٩٢/٩١ . أجريت على إثنين وأربعين عزلة بكتيرية إختبارات بيوكيماوية وفسولوجية، وتبين أن المسبب المرضي هو :

Xanthomonas campestris pv. phaseoli (Smith) Dye

وهذا المرض يتواجد في كافة مناطق الزراعة في غور الأردن.

كذلك أجريت ثلاثة تجارب، إثنين منها في الحقل المفتوح والثالثة في بيت بلاستيكي في محطة البحوث الزراعية التابعة للجامعة الأردنية في الغور الأوسط لدراسة تطور المرض وعلاقته بدرجات الحرارة والرطوبة النسبية، ولدراسة قابلية ثمانية أصناف مختلفة من الفاصولياء للإصابة الطبيعية بالمرض .

وقد دلت النتائج على ما يلي :

١ . أن المرض قد إنتشر عند درجات حرارة تراوحت ما بين ٨,٥ - ٣٩,٥ م° ورطوبة نسبية ٣٠,٦ - ٨٥,١ % . كما وجد أن معدل درجات الحرارة ٢٤ م° ورطوبة نسبية أكثر من ٥٠ % كانت الظروف الأكثر ملائمة لإنتشار المرض، كما ساعدت الأمطار على تطور وانتشار المرض .

٢ . أصيبت الأصناف الثمانية المستخدمة بالمرض في المواعيد الزراعية المختلفة، بدرجات متفاوتة . فبينما كان الصنفان Kentucky Wonder و Trophy حساسين للإصابة في التجارب الثلاث، كانت الأصناف Tema, Strike و Wade ذات مقاومة