EXPERIMENTAL RESULTS

I - Pathological studies :

A - Pathogenicity trails :

Ten isolates (I, II, III, IV, V, VI, VII, VIII, IX, and X) of motile, rod-shaped, Gram negative bacteria were isolated, on nutrient glucose agar, from 10 different field-grown plants of Brassica oleraceae L. var.capitata. cultivar Kahira Hagin showing typical head soft rot symptoms (Fig.1) .

Each of these isolates was inoculated once into the stem (by means of contaminated needle) and once into the head (by dropping the bacterial suspension into an opening artificially made at the top of the head) of Kahira Hagin cabbage. Ten plants were inoculated in each case .

All plants inoculated with any of the isolates showed head soft rot symptoms (Fig. 2), whereas control ones remained unaffected. When the pathogen was introduced into the head, the incubation period ranged from 3 to 5 days, whereas this period was 5 - 7 days upon stem inoculation.

In case of stem inoculation, the first symptoms appeared

Fig. 1 : Kahira Hagin cabbage plants, transplanted from the field, showing natural infection with head soft rot; isolate VII was secured from plant on the right .

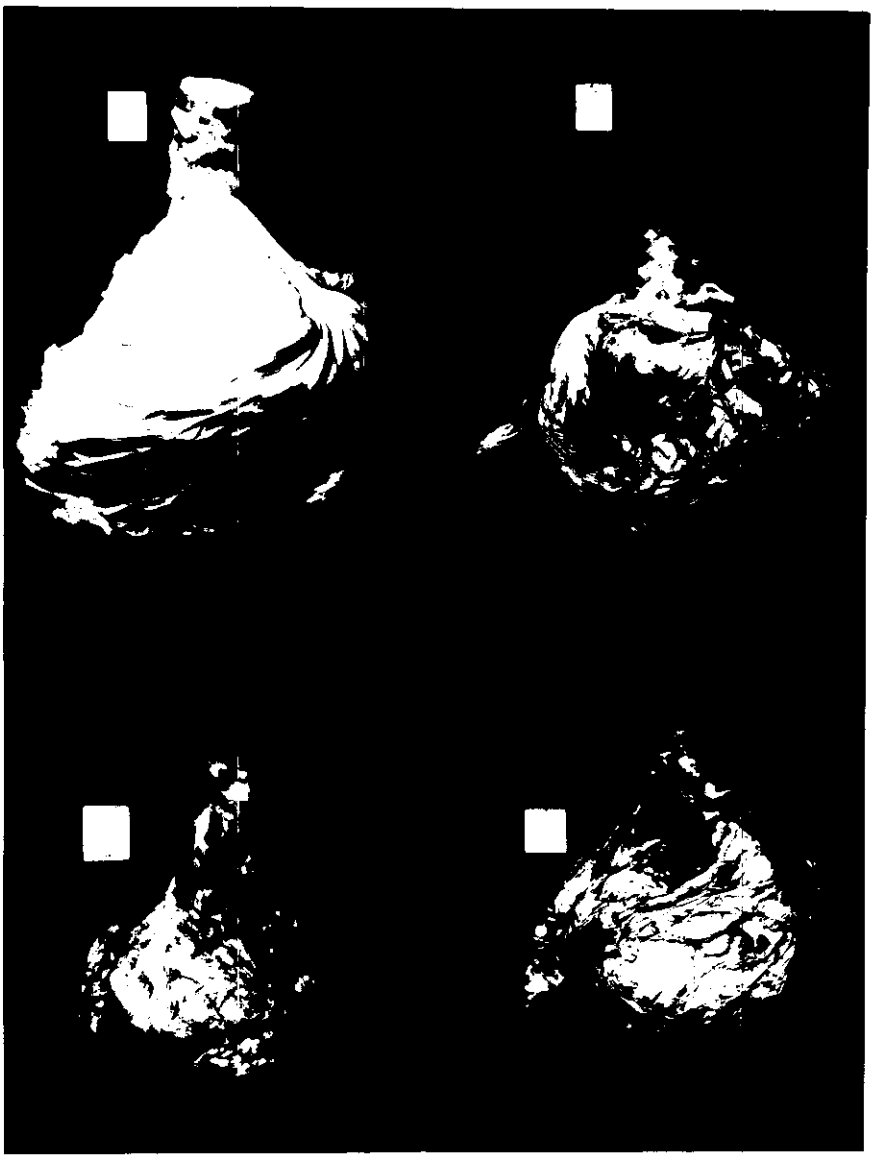

Fig. 2: Symptoms (soft rot and disintegration of tissues) incited by the isolated bacteria on inoculated heads of Brassica oleraceae L. var. capitata . 1) Healthy plant; 2, 3, 4) plants inoculated with isolates I, III, and VII, respectively .

2) Moderate isolates :

These are represented by isolates III, IV, V, and VI. Infection with these isolates was characterized by the appearance of water-soaked areas, followed by light brown discolouration of the affected areas. Later, the disease progressed to involve the whole plant head.

3) Milder isolates :

Isolates I and II showed the mildest symptoms in comparison with those incited by the above-mentioned ones. Symptoms appeared as water-soaked areas, followed by light brown discolouration, changing to dark brown at the borders, of the affected areas. In this case the disease progressed slowly and was confined to small areas .

In case of stem inoculation, with any isolate of the above groups, the discolouration of affected tissues corresponded with that appearing after head inoculation .

B - Host range :

The reactions of 12 plant species belonging to 9 families to three (I, III, VII) bacterial isolates (each representing on of the above mentioned groups) were determined in this study.

The main symptons that appeared on susceptible plants are illustrated in Table 3 and Figs. 3 - 6 .

Results could be summarized in the followings :

1) Canna plants appeared to be immune to all tested bacteria.

2) The most severe bacterial isolate according to pathogenicity tests on Kahira Hagin cabbage, i.e., isolate VII, appeared to be of wider host range in comparison with the other two tested ones. It infected 11 plant species out of 12 tested ones. This isolate infected calla and cauliflower; whereas both other isolates were not able to parasitize these plant species.

3) Isolate III was unable to infect onion, whereas isolate I infected its bulbs only, and isolate VII infected its tubular green leaves only .

4) The severity of symptons induced on susceptible plants was not a permanent feature of the isolate, in other

Fig. 3 : Rot symptoms produced on maize var. Amricani Badry after leaf inoculation with isolates VII (left) and III (center); healthy seedling right .

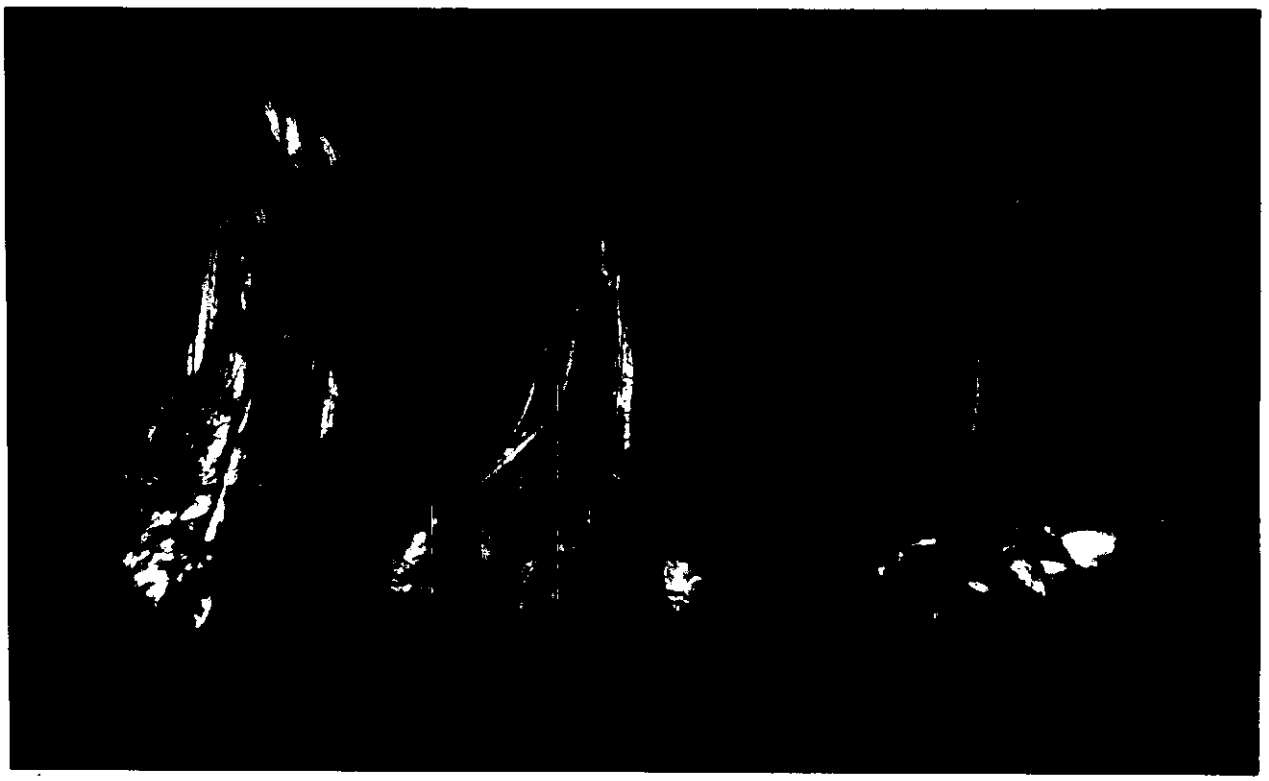

Fig. 4 : Rot symptoms produced on lettuce after leaf inoculation with isolate VII (left) and isolate III (center); healthy plant right.

Fig. 5 : Rot symptoms on leaves and roots of raddish after root inoculation with isolate VII(left) and isolate III (center); healthy plant right.

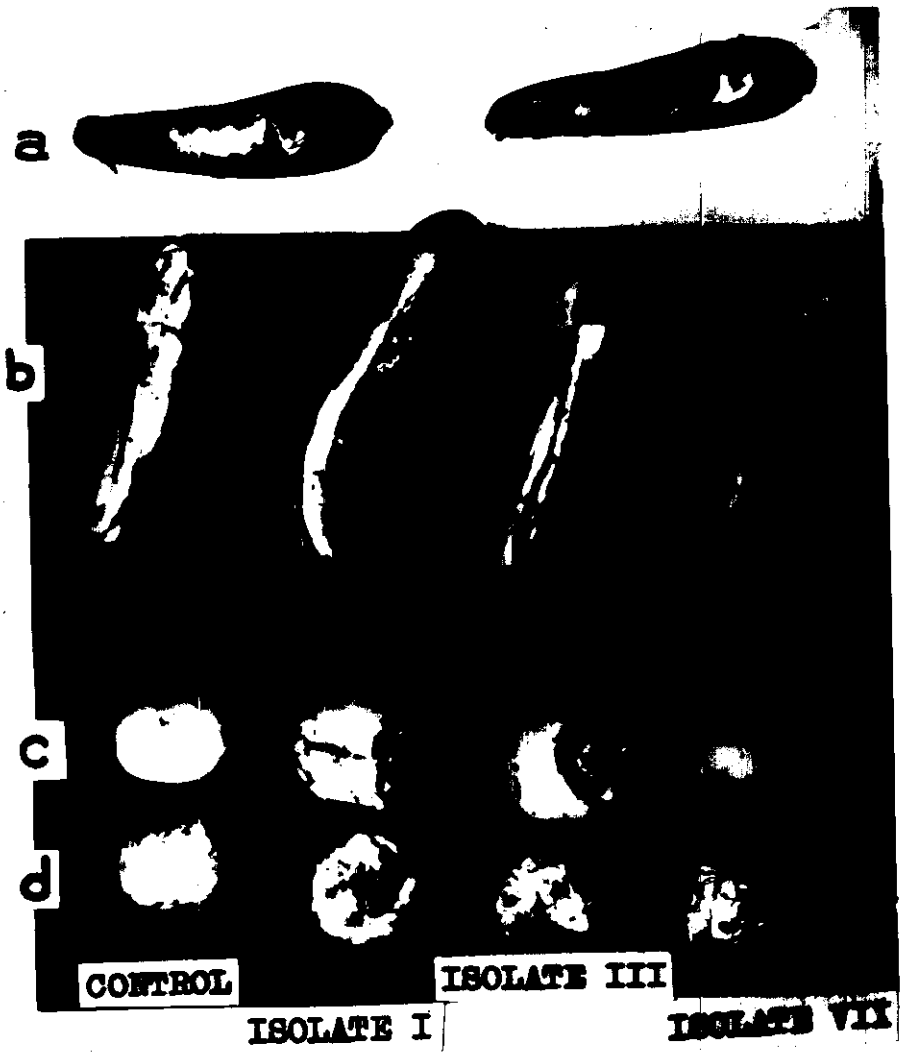

Fig. 6 : Soft rot symptoms caused by the bacterial isolates on a) fruit of Black Balady eggplant infected with isolate VII; b) Eskandarani squash fruits infected with isolates I, III, and VII; c and d) potato tubers and tomato fruits, respectively, infected with the same isolates; healthy controls left .

words isolates vary independently as regards severity of symptoms they induced on different hosts. For example, isolate VII, induced the most severe rot symptoms in lettuce, whereas in potato and squash such symptoms were incited by isolate III .

5) Generally, except in cases where calla and cauliflower were inoculated with isolate VII through leaves, symptoms took the form of soft rot .

Table 3.- Symptom expression obtained with the tested isolates on different hosts

Test plant	Site of inoculation	Disease expression		
		Isolate I	Isolate III	Isolate VII
Maise (Zea mays L. var. Amricani Beary)	Leaf	R*	R	R
	Stem	-	-	-
Calla (Zantedeschia aethiopica Spreng)	Leaf	-	-	Brown spots (R)
	Corm	-	-	
Canna (Canna indica L.)	Leaf	-	-	-
	Rhizome	-	-	-
Lettuce (Lactuca sativa L. var longifolia) .	Leaf	R^+	R^+	R^{++}
Tomato (Lycopersicon esculentum Mill var.Pritchard)	Fruit	R^+	R^+	R^+
Potato (Solanum tuberosum L. var. Alpha)	Tuber	R^+	R^{++}	(R)
Eggplant (S. melongena L. var. Black Balady)	Fruit	R^+	R^+	R^+
Squash (Cucurbita pepo L. var. Eskandarani)	Fruit	R^+	R^{++}	R^+
Onion (Allium cepa L. var. Giza 6 Improved)	Tubular green leaf	-	-	R
	Bulb	R	-	-
Cauliflower (Brassica oleraceae L. var. botrytis cultivar Orgival)	Leaf	-	-	Small silvery green spots at the sites of inoculation
	Stem	-	-	-
Raddish (Raphanus sativus L. var. Balady	Leaf	R	R	R^+
	Root	(R)	(R)	(R)
Carrot (Daucus carota L. var. sativa cultivar Chantenay	Leaf (unwounded)	-	-	R
	Root	(R)	(R)	(R)

* - : no infection; (R) : mild rot; R : moderate rot; R^+: severe rot; R^{++} : extremely severe rot .

C - Formation of hyper-sensitive reaction (HR) in tobacco leaf tissues :

In order to test the possible development of hyper-sensitive reaction (i.e., formation of necrotic area at the sites of infection), in White Burley tobacco leaf tissue, suspensions of the bacterial isolates I, III, and VII, as well as sterilized tap water (for control) were injected intercellularly at different sections of each of five leaves .

The results obtained by injecting the tested isolates into tobacco leaves are shown in Table 4. No symptoms appeared in case of inoculation with isolate I or III, whereas clear hypersensitive reaction appeared 24 hours after injecting tobacco leaves (in the mesophyll) with isolate VII. Three days later, the necrotic area became greenish violet or brown in colour .

Table 4 :- Reaction induced by bacterial isolates in the tobacco leaf three days after injection

Bacterial species	Hyper-sensitive reaction
Isolate I	- *
Isolate III	-
Isolate VII	+
Control (injection with sterilized water)	-

* - = negative reaction whereas + = positive reaction .

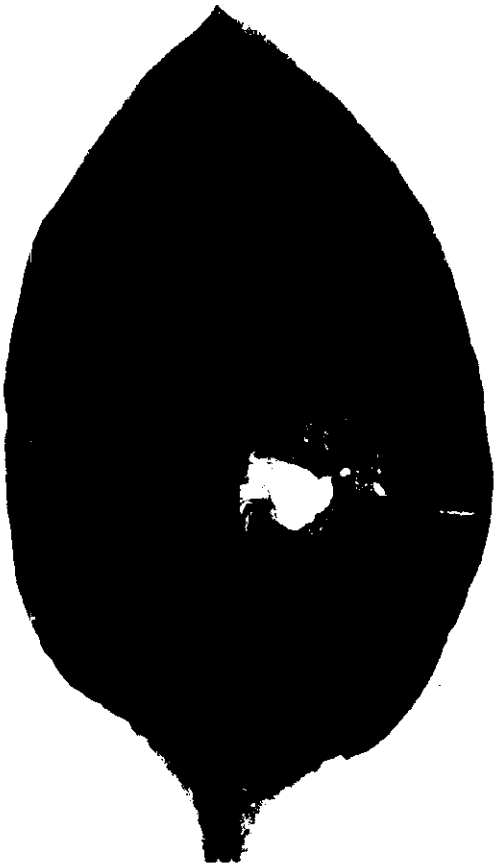

Fig. 7 : Reactions of a White Burley tobacco leaf injected by different bacteria : Left half, no reaction with isolate III; right half, hypersensitive reaction with isolate VII .

D - Determination of infection route :

Kahira Hagin cabbage plants were inoculated with isolate VII by means of root, stem, or leaf inoculation techniques (as described under the section of MATERIALS AND METHODS) in order to reveal the main route of infection by this head soft-rot pathogen .

Neither root nor stem inoculation techniques had given head rot symptoms during the experimental period which extended up to 4 weeks after inoculation. Only leaf inoculation technique could produce head rot symptoms on leaves within 7 - 10 days .

The above results indicate that leaves are the main route for natural infection with isolate VII .

E - Interaction between head soft-rot pathogens :

a - On media (in vitro) :

Isolates I, III and VII were tested against each other for their possible antagonistic action. For this purpose perpendicular streaking method (used by El-Helaly et al., 1971) was used as follows .

After 4 days growth on nutrient-glucose agar (of the isolate to be tested for antagonistic action), another isolate was perpendicularly streaked on the same plate and the growth inhibition zone was checked after 3 days incubation at 30 °C .

The obtained results (Fig. 4) show that only isolate VII has inhibitory effect against both other ones .

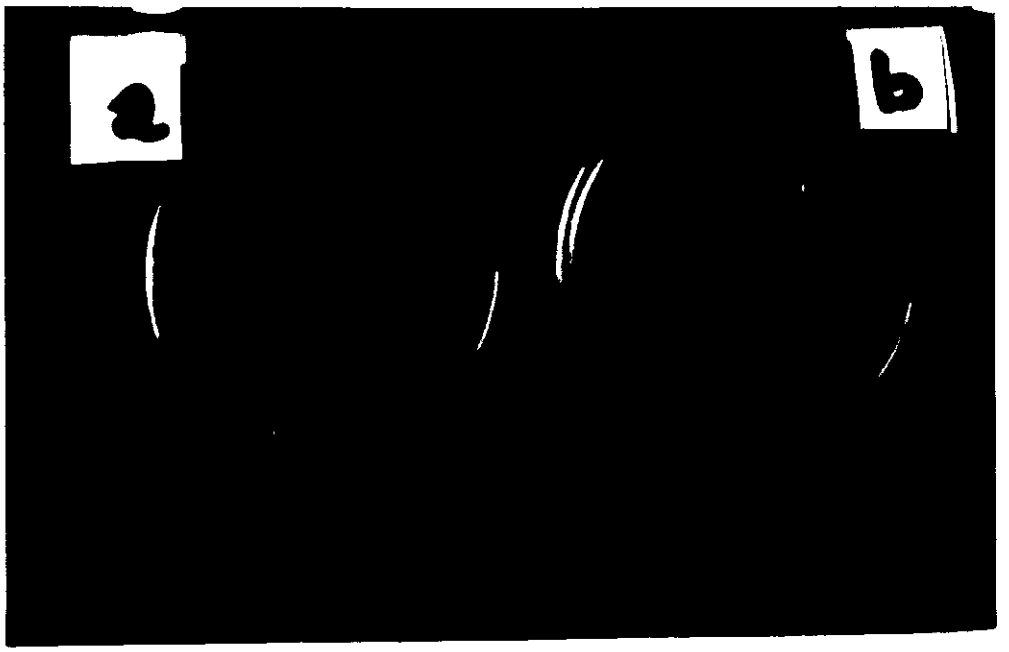

Fig. 8 : Antagonistic reaction of isolate VII against isolates I (a) and III (b) <u>in vitro</u> .

b - Interference between bacterial isolates in green-house tests :

In order to study the possible interaction between isolates when simultaneously inpculated to the host, the effect of inoculating cabbage leaves with 1 : 1 mixtures of certain isolates, as well as with single isolates, on development of disease symptoms was determined. Four days after inoculation the diameter of rotted areas in different treatment was measured .

The results are shown in Table 5. It is clear that inoculation with mixtures of isolates I and VII or of III and VII resulted in a reduction of bacterial rot symptoms as compared to inoculation with any of the three involved isolates when tested alone. This indicates that in mixtures the isolates are not synergistic but slightly antagonistic.

Table 5 .- Effect of inoculating leaves of Kahira Hagin cabbage with single bacterial isolates or with their mixtures on development of rot symptoms

Isolates	Diameter of rotted area (cm), 4 days after inoculation
I + VII	1.42
III + VII	1.45
I	1.75
III	2.10
VII	2.80
None (sterilized water as control)	0.00

F - Insect transmission of infection with isolate VII :

An attempt to transmit infection with isolate VII by the cotton leaf worm (Spodoptera littoralis, Biosd.) and the cabbage aphid (Brevicoryne brassicae L.) was carried out. Insects, after being contaminated with the bacterial isolate, were transferred to feed on detached and singly caged leaves of Kahira Hagin cabbage .

Results are summarized in Table 6. Rotted areas about 3 - 5 cm in diameter appeared within 3 - 5 days in all tests with mechanical inoculation or with contaminated cotton leaf worms. No rot developed in tests with uncontaminated leaf worms. The cabbage aphids were not able to transmit the rot disease under the conditions of the experiment .

Table 6 .- Mechanical and insect transmission of infection with isolate VII

Treatment	No. of infected leaves out of 10 treated ones
Mechanical inoculation	10
Contaminated *Spodoptera littoralis* Biosd.	10
Uncontaminated *S. littoralis* Biosd.	0
Contaminated *Brevicoryne brassicae* L.	0
Uncontaminated *B. brassicae* L.	0

2 - Morphological and cultural characters of the organisms :

The following description of the head soft-rot organisms of Brassica oleraceae L. var.capitata cultivar Kahira Hagin are based on studies of characters of the 10 isolates. According to these studies, it was possible to classify the ten isolates into three groups :

a) Group 1 includes isolates I and II .

b) Group 2 includes isolates III, IV, V, and VI .

c) Group 3 includes isolates VII, VIII, IX and X .

All isolates in each group appeared to have similar characters .

A - Morphological characters :

Light microscopic examination revealed the following description :

Form of cells : All isolates are short rods, nonspore-forming, with rounded ends. Isolates of groups 1 and 2 mostly occur as single rods, whereas those of groups 3 are frequently in chains of up to 3 cells .

Size of cells : Using ocular micrometer, the cell size

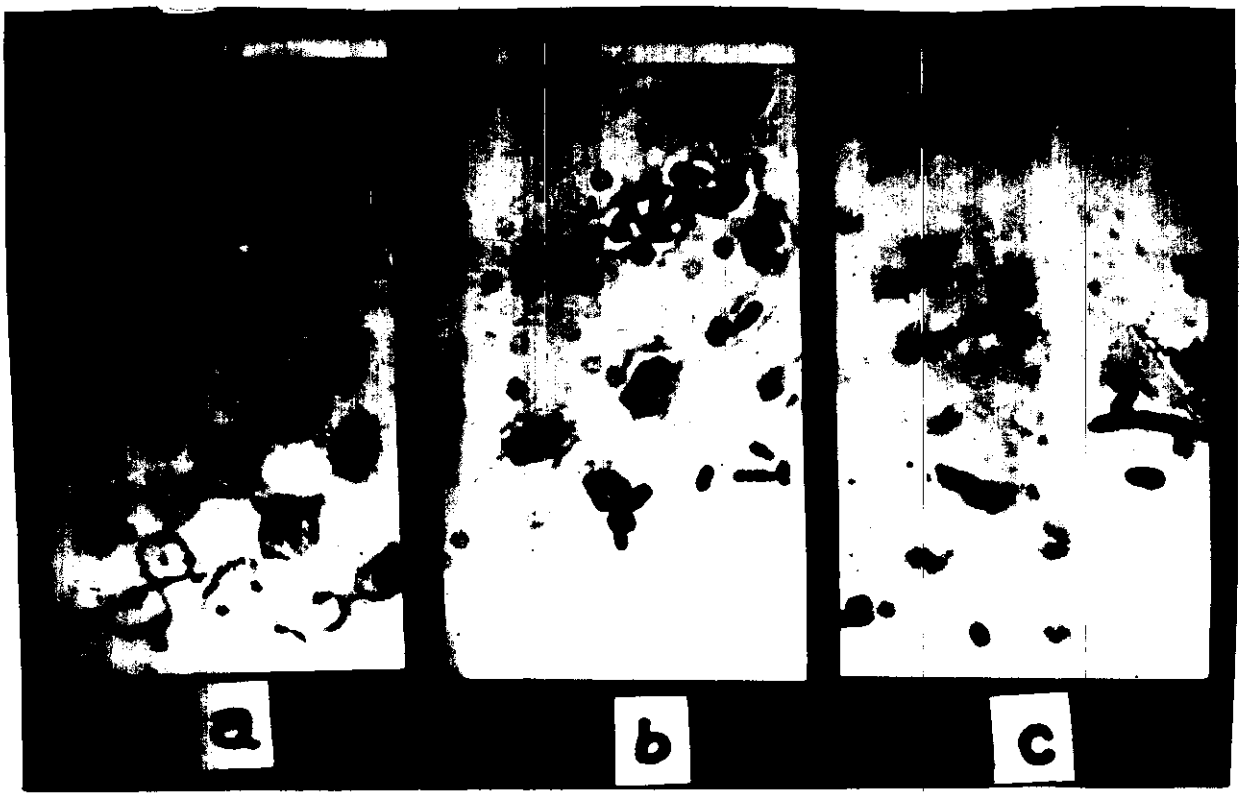

Fig. 9 : Photomicrographs of the isolated bacteria .
a) Isolate I, b) isolate III,c) isolate VII.

of isolates of the three groups was approximately as follows :

Group 1 : 0.6 to 0.7 by 0.8 to 0.9 µ .

Group 2 : 0.5 by 0.8 to 0.95 µ .

Group 3 : 0.4 to 0.5 by 1.4 to 2.2 µ .

Motility : Isolates of all groups are motile; group 1 and 2 by 2 - 6 peritrichous flagella, and group 3 by 1 - 2 polar flagella .

Staining : All isolates of the three groups are Gram nagative and not acid fast .

Capsulation : Isolates of groups 1 and 2 are surrounded by sline layer, but those of group 3 are not.

B - Cultural characters :

Cultural characters of the bacterial isolates were studied on different solid and liquid media .

a - Growth on solid media :

1) Nutrient-glucose agar :

All isolates grow well on this medium .

Colonies of groups 1 and 2 are creamy white, opalescent, convex with entire margins. Colonies are round, appearing

after 24 hrs at 30 °C, 2 - 3 mm in diameter, becoming 3 - 5 mm within 72 hrs. Colonies are watery, changing to slimy and opaque with age .

Colonies of group 3 are dirty white, opalescent, smooth, slightly raised with regular margin becoming irrigular in old cultures. Colonies are irrigularly round, appearing after 24 - 48 hrs at 30 °C, 1 - 2 mm in diameter, becoming 2 - 3 mm within 72 hrs. They are watery, changing to some what slimy; transparent, changing to yellow, then to green on day 3 of inoculation. A soluble fluorescent green pigment is secreted into the medium within 3 - 5 days; with age the pigment changes to dark brown .

2) Nutrient-glucose agar containing 0.3 % yeast extract :

On this medium growth of all isolates is rapid. Colonies of 1 - 3 mm . diameter appear within 24 hrs and reach 3 - 5 mm within 72 hrs. Colonies of all isolates have centers darker than margins. Isolates of group 3 produce fluorescent pigment after 36 - 48 hrs .

3) Nutrient agar medium :

Colonies are comparable to those obtained on nutrient glucose agar but growth was poorer .

4) Potato dextrose agar :

Isolates of groups 1 and 2 are moderate in growth. Colonies are creamy white, translucent and slightly raised, ranging from 1 to 2 mm in diameter after 24 hrs and from 3 - 4 mm after 72 hrs .

Growth of group 3 isolates is very poor or absent, and no pigmentation is observed .

5) Glucose asparagine agar :

Growth of all isolates is generally well .

Colonies of groups 1 and 2 are round, convex with entire margin. Colonies are grayish white, opalescent becoming semi-opaque with age. Colonies of group 3 are round, large (3 - 4 mm in diameter within 7 days), slightly raised with entire margin. Colonies are white produce a fluorescent green, water solubale, pigment .

b - Growth in liquid media :

1) Bouillon glucose :

Good growth appears after 48 hrs at 30 °C. In all cases, a fine sediment and a clear pellicle are formed, except with group 2 isolates which can not form pellicle .

When yeast extract is added to the latter medium, better growth of all isolates is observed after 48 hrs. In all cases, a thick pellicle is formed, and a considerable amount of viscous (isolates of groups 1 and 2) or unviscous (group 3) precipitate is observed at the bottom .

2) Nutrient broth :

Poor growth appears for isolates of groups 1 and 2 after 48 hrs at 30 °C. A moderate amount of viscous sediment is formed but no pellicle is observed .

Growth of group 3 isolates is well but neither sediment nor pellicle is observed .

3) Glucose asparagine :

Good growth is observed after 48 hrs. All isolates form pellicle except those of group 2. A fine sediment is formed in cases of groups 1 and 2, while it is moderate in case of group 3 isolates .

In all above mentioned liquid media, isolates of group 3 only could produce a fluorescent green pigment .

4) Asparagine-mineral solution :

In this medium poor turbidity is observed within 48 hrs. The growth becomes thick with age and the cultures gradually turn alkaline. A fine sediment is formed in cases of groups 1 and 2, while it is moderate in cases of groups 3. All isolates form pellicle except those of group 2 .

3 - Nutritional and biochemical characters :

All tests reported in this section were carried out on the 10 bacterial isolates. In all cases, results obtained with the isolates of each group (1 , 2, or 3) were similar.

A - Effect of different media on bacterial growth :

Different solid media, adjusted to pH 7, were prepared. Media were inoculated, by streaking with uniform loops of a standard bacterial suspension, then incubated at 3 °C for 3 days . Bacterial growth was visually evaluated .

The obtained results (Table 7) indicate that nutrient-glucose-yeast extract agar (NGYA) followed by nutrient -

Table 7 .- Effect of different solid media on bacterial growth as determined visually

Group no.	Isolate no.	Medium (NGYA)	(NGA)	(NA)	(GAA)	(PDA)
1	1	++++*	+++	++	++	+
	2	++++	+++	++	++	+
2	3	++++	+++	++	++	++
	4	++++	+++	++	++	++
	5	++++	+++	++	++	++
	6	++++	+++	++	++	++
3	7	++++	+++	+++	++	-
	8	++++	+++	+++	++	-
	9	++++	+++	+++	++	-
	10	++++	+++	+++	++	-

* The intensity of growth is indicated by the number of + signs; - indicates absence of growth .

glucose agar (NGA) were the best media for growth of all isolates. Nutrient agar (NA) came next to the above media in this respect; but growth of isolates of group 3 on this medium was better than that of all other isolates. Growth of all tested isolates on glucose-asparagine agar (GAA) was moderate, whereas it was moderate (isolates of group 2), scanty (isolates of group 1), or even absent(isolates of group 3) on potato dextrose agar (PDA) .

B - Utilization of different carbon compounds :

a - Sugars, alcohols, starch, and organic salts :

Different sugars, alcohols and organic salts, as well as starch were tested as sole carbon sources by incorporation in basal solution. Data were taken after 48 hrs and up to 3 weeks of incubation at 30 °C .

It will be remembered that all isolates of each of the three tested groups behaved similarly .

Data obtained are presented in Table 8, which is self-explanatory. Generally, results point out to the following :
1 - As regards utilization of different carbon compounds, isolates of group 1 and 2 differ slightly from each other but considerably from those of group 3 .

2 - All isolates of groups 1 and 2 are able to produce acid and gas from many of the tested compounds (particularly sugars), whereas those of group 3 produce acid but no gas from some of these compounds (mainly sugars also) .

3 - Group 1 and group 2 isolates produce acid but no gas from sodium citrate, sodium tartarate, salicin and aesculin, whereas group 3 isolates were not able to produce neither acid or gas from these compounds. In case of sodium citrate, isolates of group 3 changed the medium pH to alkalinity .

4 - Differences between isolates of group 1 and 2 can be summed up in the following : a) isolates of group 1 could utilize sorbose (producing acid and gas) but not dulcitol, whereas with isolates of group 2 the reverse is true, b) from lactose and manitol, acid and gas were produced by group 1 isolates and acid but not gas by group 2 isolates, and c) isolates of group 2 were not able to utilize ethanol but in case of group 1 isolates weak production of acid occurred .

b - Miscellaneous :

All isolates were not able to hydrolyse starch (in starch hydrolysis medium, Manual of Microbiological Methods, 1957) or to decompose cellulose (filter paper). Potato slices were

disintegrated possibly due to the action of pectolytic enzymes but group 3 was less active in this respect .

C - Nitrate reduction :

All isolates reduced nitrates to nitrites .

D - Gelatin liquefaction :

Gelatin is liquefied. However, isolates of different groups considerably vary as regards degree of activity; those of group 3 are highly active and show positive reaction within 24 hrs, whereas those of group 1 and 2 are feebly active and showed weak reaction after the elapse of 3 weeks .

E - Hydrogen sulphide production :

Isolates of groups 1 and 2 produced hydrogen sulphide, whereas those of group 3 could not .

F - Indol formation :

Indol was not detectable in all cases .

G - Voges-Proskaur (V.P) and methyl red (M.R) tests :

Acetyl methyl carbinol (acetoin) is produced (V.P. posi-

tive) after 48 hrs growth at 30 °C by isolates of both 1 and 2 groups. These isolates are negative to M.R test after 2 - 5 days growth at same temperature. Isolates of group 3 are negative to V.P. test and positive to M.R. test .

H - Action on litmus milk :

Cream-free litmus milk gradually became alkaline and turned deep blue within 3 days in case of group 3, while it became acid (red) in cases of group 1 or 2. Four days later, the blue or red colour was completely reduced. No clotting was observed and the milk cleared off slowly, becoming translucent within 3 weeks .

I - Lipolytic action :

Nutrient agar medium containing 0.2 % cotton seed oil was plated. Medium was inoculated at the center with a loopful of standard suspension of the isolate to be tested. Inoculated plates were incubated at 30 °C. Lipolytic action was checked at three intervals, i.e., 2, 7, and 21 days after incubation.

Results showed that only isolates of group 3 are lipolytic .

4 - Conditions for growth :

A - Aerobiosis :

The group 3 isolates are aerobic as the growth in nutrient-glucose agar shake cultures was invariably superficial, while groups 1 and 3 isolates are facultative aerobic .

B - Temperature relations :

Eight isolates (No. 1 to 8) were tested in this experiment. Bouillon glucose tubes(10 ml/tube)were inoculated (one loopful per tube) with a standard water suspension (10^8cell/ml) of the isolate to be tested, and were then incubated for 48 hrs at 5, 10, 15, 20, 25, 30, 35 and 40 °C. Four replicates were used for each treatment. The rate of growth was measured after 3 days by determining the optical density using a universal colourimeter with a green filter.

The obtained results (Table 8 and Fig. 10) show that growth of isolates of each particular group was almost similarly affected by the temperature at which cultures were incubated. However, isolates of different groups were variably affected by this temperature .

Table 9 .- Effect of temperature on growth of bacterial isolates

Group no.	Isolate no.	Average % of relative absorption after 48 hrs incubation at :							
		5	10	15	20	25	30	35	40°C
1	I	0.0*	8.5	31.75	56.5	64.0	81.0	80.5	0.4
	II	0.0	8.75	35.0	57.5	65.5	80.0	79.0	0.0
2	III	0.75	18.75	34.0	56.25	65.0	67.0	68.5	0.0
	IV	0.25	14.0	33.5	54.5	60.75	68.5	66.0	0.0
	V	0.40	10.0	26.0	61.75	65.25	64.5	64.25	0.0
	VI	0.50	9.0	16.75	62.5	64.0	68.0	65.5	0.0
3	VII	0.0	0.0	0.0	32.75	41.5	59.0	58.25	52.25
	VIII	0.0	0.0	0.0	36.75	40.75	55.25	52.5	54.25

* Data represent averages of 4 replicates of original readings in a universal colourimeter. The original readings were related to : 0 percent absorption of the uninoculated tubes held at the same temperature conditions .

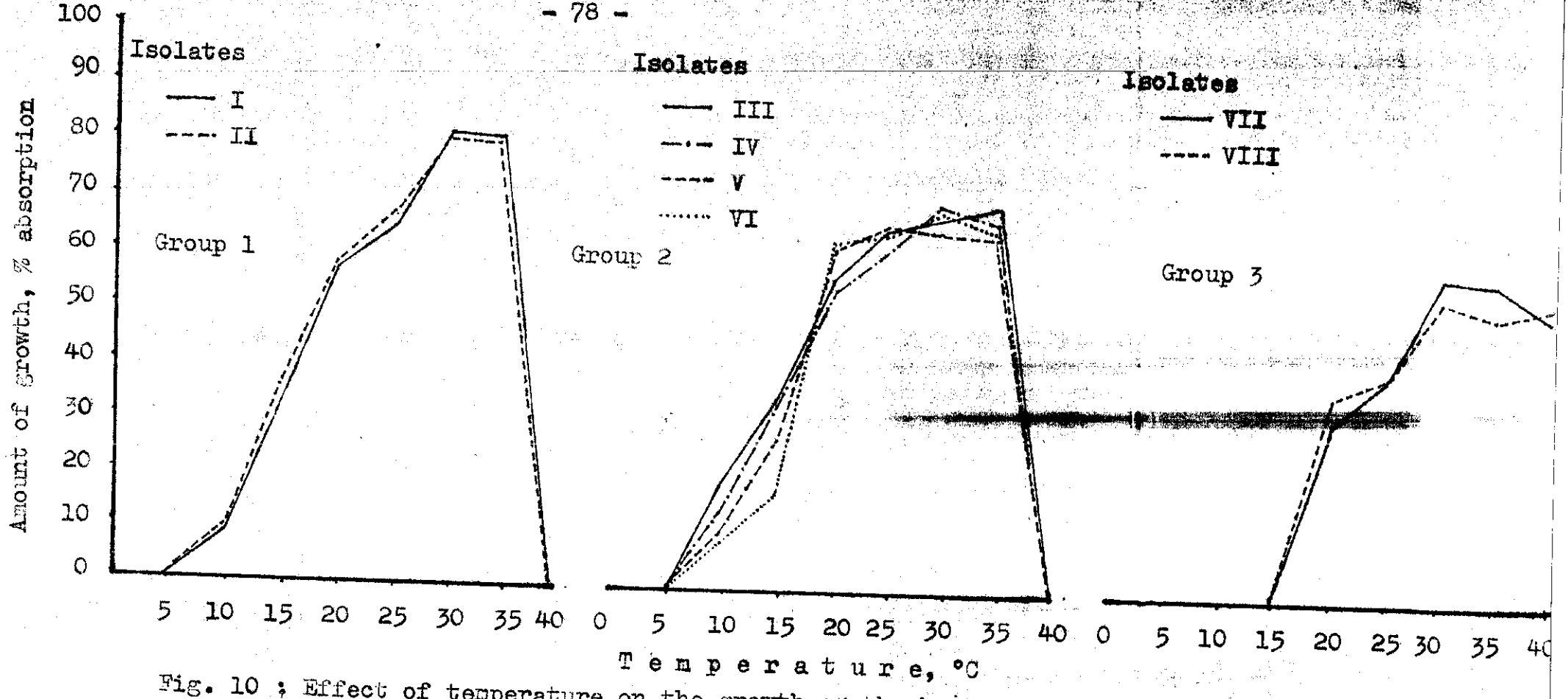

Fig. 10 ; Effect of temperature on the growth or the bacterial isolates

As shown in Table 10, the minimum, optimum, and maximum temperatures were : 5-10, 30 and 35 - 40 °C (group 1); < 5, 30, and 35 °C (group 2); 15 - 20, 30 - 35 and > 40 °C(group 3).

Table 10 .-Minimum, optimum, and maximum temperatures of bacterial isolates

Isolates of group :	Minimum	Optimum	Maximum temp.(°C)
1	5 - 10	30	35 - 40
2	< 5	30	35
3	15 - 20	30 - 35	> 40

C - Thermal death point :

The same 8 isolates used in the preceeding test were employed in this experiment. Thermal death point was determined by heating 1-cm samples, contained in thin-walled test tubes with internal diameter of about 7 mm, of a dense water suspension of the isolate to be tested at certain temperatures for 10 minutes. Two series of experiments were carried out; in the first series the temperatures ranged from 40 to 60 °C, in 5 degree steps, whereas in second series the range was 50 to 60 °C, in one-degree steps. At the end of heat treatment, the

tubes were immediately cooled under tap water. Four replicates were used in each treatment. Viability of the bacteria was checked by transferring loopfulls of the treated suspension to nutrient glucose agar slants and incubation at 30 °C.

The thermal death point of all isolates of both 1 and 2 groups appeared to be 55 °C, whereas that of group 3 isolates was 57 °C .

D - Effect of pH value on growth :

Isolates I, III and VII (representative of group 1, 2 and 3,respectively) were selected for this experiment. Nutrient broth tubes adjusted to certain pH values (4.6, 6.2, 6.8, 7.8 and 9.4), using potassium phosphate buffer, were inoculated with uniform loopfuls of standard bacterial suspensions. Four replicates were used for each isolate. Relative amounts of growth were determined photometrically after 2 days incubation at 30 °C, using a universal colourimeter (Lichtelektrisches Kolorimeter Model VII) .

Data obtained and presented in Table 11 and Fig. 12 point out to the following :

All tested isolates could grow within a wide range of pH values (from 6.2 to 9.4). Neutral to alkaline range of pH (6.8 - 7.8) was more favourable for isolates 1 and 3 (representatives of groups 1 and 2, respectively). Isolate 7 (representative of group 3) aloso could grow well at this pH range, but its best growth occurred at pH 6.2. None of isolates could grow in the extremely acid medium (pH 4.6) .

Table 11 .- Effect of pH value on the growth of bacterial isolates

Bacterial isolate	Average % of relative absorption after 2 days growth in bouillon-glucose medium adjusted to the indicated pH;				
	4.6	6.2	6.8	7.8	9.4
I	0.0*	4.0	6.0	8.75	4.5
III	0.0	2.5	5.0	4.75	3.0
VII	0.0	18.2	16.5	16.0	12.0

* Data represent averages of four original readings (four replicates) in a universal colourimeter. The original readings were related to : 0 percent absorption of the uninoculated tubes having the same pH value .

Isolates

I

20

10

0

5 - Enzymatic activities :

A - Pectolytic activity :

Tests for liquefaction of pectate gel, and production of pectin methylesterase, pectin polygalacturonase, and protopectinase were carried out to determine and compare the pectolytic activities of the organisms under investigation .

a - Liquefaction of pectate gel :

Data obtained (as shown in Table 13) indicate that isolate VII is a strong liquefier of pectate, whereas both of isolate I and III gave a weak reaction. Control plates remained unaffected during the experimental period that extended up to 5 days .

b - Pectin methylesterase (PME) :

The organisms to be tested were grown in nutrient broth at 30 °C.

A preliminary trial using the test solution described under the section of MATERIALS AND METHODS was carried out .

Table 13 shows the results and the time required by cultures to produce PME. All of the tested bacterial isolates

gave a clear reaction after 2 days incubation in the growth medium (nutrient broth). Isolate I was the most active in this respect .

c - Pectin polygalacturonase (PG) :

When the pectin agar plates were flooded with hydrochloric acid 5 N, a narrow clear zone surrounded by a precipitate of pectic acid was obtained with each of isolates I and III, whereas in case of isolate VII a wider (3 - 4 mm) clear zone appeared .

When the plates were flooded with calcium chloride (10%), isolate VII only gave a positive reaction (a clear zone, 2-3 mm wide, surrounded by a precipitate, about 3 - 5 mm in width). All isolates gave negative reaction (no precipitate or clear zone) when flooded with 5 % lead acetate.

Using information given by Dingle et al. (1953) the reactions expected from flooding the pectin agar plates with hydrochloric acid, calcium chloride, or lead acetate were suggested by Dye (1960) to be as shown in Table 12.

Thus, the obtained results (see Tables 12 and 13) clearly demonstrate that all bacterial isolates are active in hydro-

lyzing low methoxyl pectin (i.e., possess enzymes of the polygalacturonase type) .

The isolates, however, differed in the degree of hydrolysis they caused. Isolate VII is the most active since it hydrolyses low methoxyl pectin to lower uronides and/or galacturonic acid; isolate III followed by isolate I came next to it (in their case, hydrolysis does not proceed to the production of uronides) .

d- Protopectinase (PP)

Data obtained and presented in Table 12 and Fig. 12 show that all tested bacterial isolates produce protopectinase. When potato slices (var. Alpha) were used, isolates I and III induced maceration (which resulted in dropping of the unsupported end of the slices to the glass) within 12 - 15 hrs, whereas isolate VII gave the same result 15 - 24 hrs after inoculation.

When lettuce stem slices were used, all tested bacterial isolates macerated then within 12 - 15 hrs .

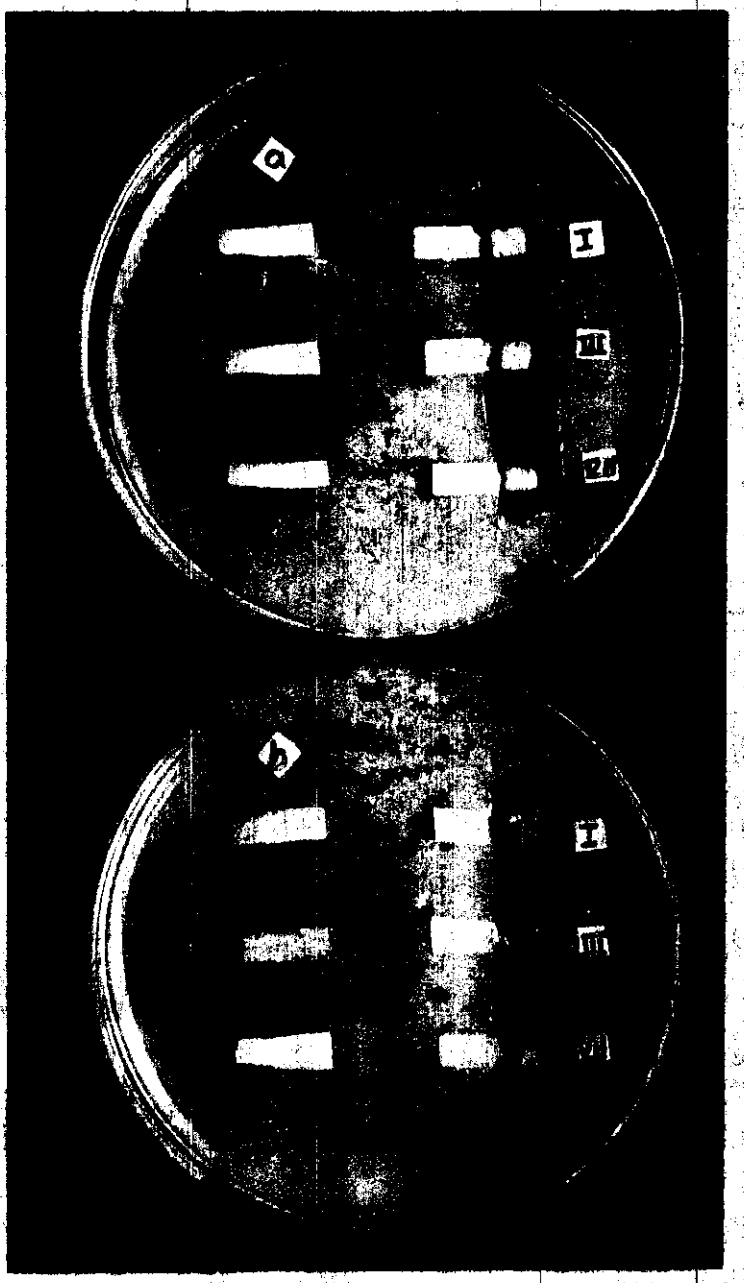

Fig.12: a) The cantilever potato slices method for testing the protopectinase activity of bacterial isolates (I, III, and VII). b) Lettuce stem slices used for the same purpose. Left : slices uninoculated .

B - B-glucosidase activity :

Plates of nutrient agar medium containing 1 % (W/V) aesculin and 0.05 % (W/V) ferric citrate (Rushdi et al., 1972) were inoculated with standard inocula prepared by adding equal volumes of sterile distilled water to 2-day-old cultures of each isolate. Each plate was inoculated in the center with a loopful from single isolate. Five plates were used for each isolate. After 3 days incubation at 30 °C, the diameter of the black zone (resulting from reaction between ferric citrate and the phenol liberated from the aesculine glucoside) around the colonies was measured.

Results presented in Table 14 and Fig. 13 show that both isolates I and III could produce B-glucosidase, whereas isolate VII could not. It is to be mentioned that the highest B-glucosidase activity associated the less virulent bacterial strain (i.e. isolate I) .

Table 14 .- B-glucosidase activity of the bacterial isolates

Isolate no.	Average diameter of the black zone around the colonies after 3 days incubation at 30°C(in mm)
I	55
III	50
VII	00

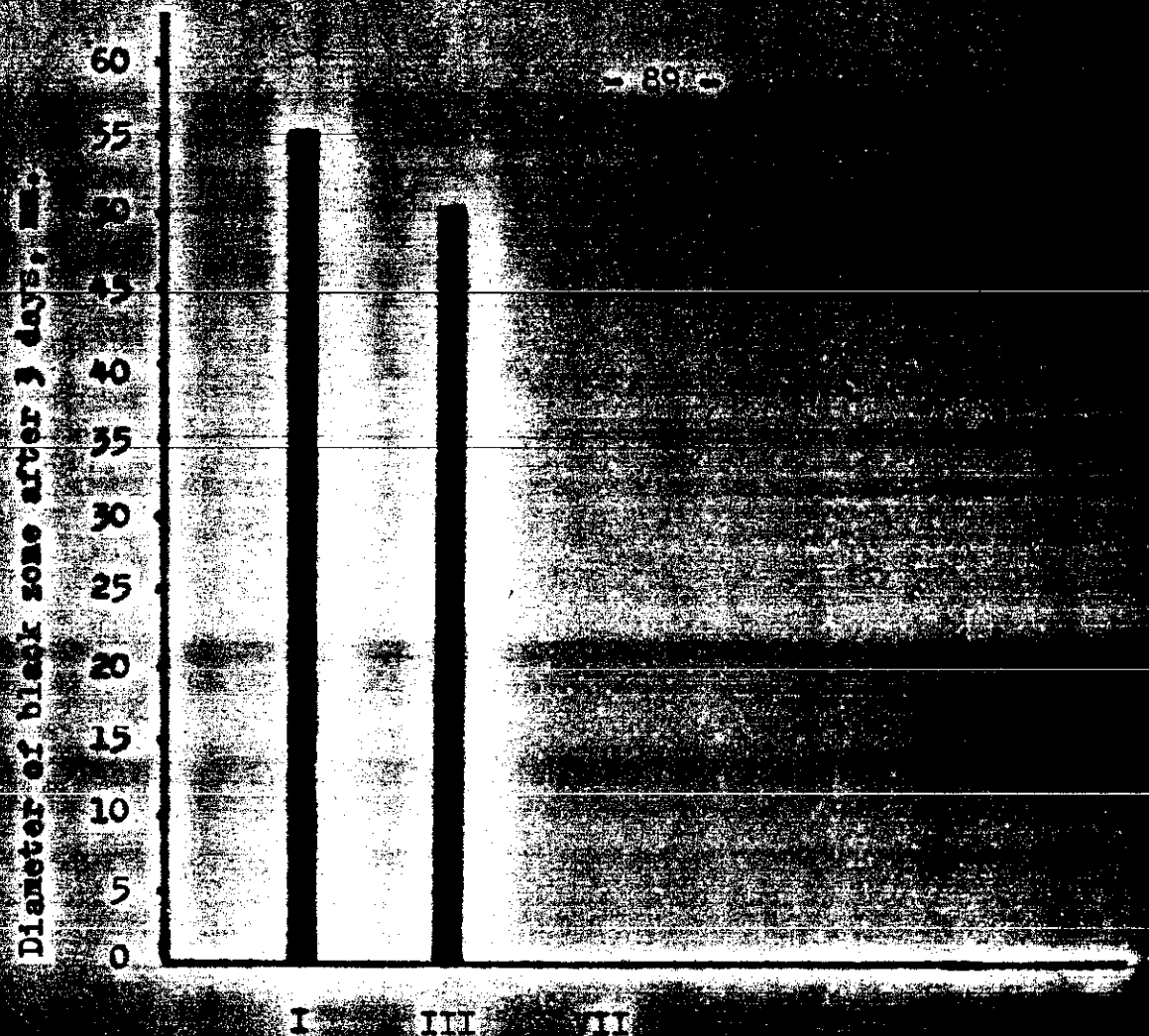

Fig. 13 : B-glucosidase activity of bacterial isolates

C - Catalase activity :

Quantitative test for catalase activity (Collowick and Kaplan, 1955) of the three bacterial isolates was carried out by adding 2 ml of standard cell suspension (16 mg dry weight/ml) of the isolate to be tested to 4 ml of 0.6 % hydrogen peroxide. Flasks containing the mentioned mixture were kept in water bath at 30 °C and the reaction was stopped after intervals of 5, 10, or 15 minutes by adding 10 ml of 1 N H_2SO_4. The decomposition of H_2O_2 was measured by titrating the remaining substrate with 0.01 N potassium permanganate. Four replicates were used for each isolate .

From the data presented in Table 15 and Fig. 14, it can be seen that all isolates were able to produce catalase. However, isolate VII had higher catalase activity than both others, which showed almost same activity .

Table 15 :- Catalase activity of bacterial isolates

Isolate no.	Milligrams hydrogen peroxide decomposed after 5	10	15 minutes
I	3.06	4.54	5.36
III	2.60	3.70	4.88
VII	10.60	12.00	12.00

6 - Toxicity of phenols and quinones to the bacterial isolates :

Pyrocatechol, resorcinol, hydroquinone, and p-benzoquinone were tested for toxicity against the bacterial isolates I, III, and VII .

Aqueous stock solution of the compounds were sterilized through Sietz filter, then predetermined amounts of the solutions were aseptically added to previously autoclaved nutrient-glucose agar medium to provide the desired final concentrations of each compound. The treated and control (without compounds) media were plated. Plates were streaked with uniform loops from standard inocula of the bacterial isolates. Inoculations were performed in quadruplicates. Plates were incubated at 30 °C for 4 days .

Data obtained and presented in Tabe 16 point out to the following :

1 - The most effective compound in inhibiting the growth of all isolates was p-benzoquinone, at 125 ppm of this compound none of the three isolates was able to grow.

2 - Hydroquinone and pyrocatechol followed p-benzoquinone as regards effeciency to inhibit bacterial growth; isolates I

Table 16 :- Toxicity of phenolic compounds and quinones to the bacterial isolates

Compound	Concentration, ppm	Isolates		
		I	III	VII
Pyrocatichol	125	+*	+	-
	250	-	-	-
	500	-	-	-
	1000	-	-	-
Hydroquinone	125	+	+	-
	250	-	-	-
	500	-	-	-
	1000	-	-	-
Resorcinol	125	++	++	+
	250	+	+	(+)
	500	(+)	(+)	-
	1000	(+)	(+)	-
	1500	-	-	-
p-benzoquinone	125	-	-	-
Control		++	++	++

* ++ Abundant growth. + Moderate growth. (+) Scanty growth. - No growth.

and III were unable to grow at 250 ppm concentration of these compounds, and isolate VII even at 125 ppm .

3 - Resorcinol also showed inhibitory effect, but it came next to the above compounds .

4 - Isolate VII was the most sensitive one to all tested compounds. It showed no growth at 125 ppm p-benzoquinone, pyrocatichol, or hydroquinone and at 500 ppm resorcinol, whereas other isolates showed moderate or scanty growth at the indicated levels of the latter three compounds .

7 - Control experiments :

A - Laboratory tests :

Fungicides and antibiotics listed in Table 2 were tested at different concentrations against isolates I, III, VII in nutrient-glucose agar medium adjusted to pH 7. Calculated aliquots from stock solution or suspension of the funigicide or antibiotic to be tested were added aseptically to the medium to give the desired final concentration. Antibiotics (pure) were tested at concentrations of 10, 25, 50, and 100 ppm, whereas fungicides were tested at 200, 300, 400, 500, and 1000 ppm of the active ingredient . Control medium was treated with distilled water. Standard bacterial suspension of the isolates were prepared in sterile distilled water from 2-day-old nutrient-glucose agar cultures. Plates of different treatments (4 per treatment) were streaked with the pathogen, and then incubated at 30 °C for 3 days .

Data presented in Table 17 show the minimal dose of the tested compounds that caused complete inhibition of the growth of the tested bacteria. It is to be seen that :

1 - The tested antibiotics were substantially more effective in inhibiting bacterial growth than any of the tested fungi-

cides. Oxytetracycline chloride caused complete inhibition of isolate I and III at the lowest concentration used (10 ppm) and of isolate VII at 25 ppm, whereas Neomycin sulphate incited such effect to the former isolates at 25 ppm and to the latter at 50 ppm .

2 - Dithane Z-78 followed the above compounds in efficiency to inhibit bacterial growth .

3 - Copper oxychloride was also highly effective but came next to Dithane Z-78 .

4 - Benlate, Topsin, and Terracolor were the least effective compounds in inhibiting bacterial growth; none of them caused complete inhibition of any isolate even at its highest concentration used (1000 ppm) .

Table 17 .- Toxicity of some fungicides and antibiotics to the bacterial isolates

Compounds	Minimal concentrations(ppm of active ingredient) affecting complete inhibition of tested isolates		
	I	III	VII
Fungicides :			
Dithane Z-78	300	200	500
Copper oxychloride	500	200	500
Terracolor	*	*	*
Benlate	*	*	*
Bavisitin	*	*	*
Topsin	*	*	*
Antibiotics :			
Oxytetracycline chloride	10	10	25
Neomycin sulphate	25	25	50

* Complete inhibition was not acheived at 1000 ppm .

B - Chemical control of disease in the field :

Dithane Z-78, Copper oxychloride, Oxytetracycline chloride and Neomycin sulphate were tested for controlling the head soft -rot of Brassica oleraceae L. var. capitata plants incited by isolate I or VII of the pathogen .

Field-grown Kahira Hagin cabbage plants were given only one spray with a suspension (or solution) of the tested compound or with sterilized water for the control. Tests included three different times of applications (3 or 7 days before inoculation, or one day after inoculation) and three different levels (shown in Table 18 and 19) of each compound. Leaf inoculation technique was employed by spraying a standard suspension of the desired inoculum on a round scratched area, 15 mm in diameter. Fifteen replicates (each consisting of one plant) were used in each treatment. Data were recorded, in the form of diameter of rotted area, 5 days after inoculation. The experiment was replicated in two successive years (1974 & 1975).

Data obtained and presented in Table 18 clearly show that all the antibiotics and fungicides tested in this experiment are effective in decreasing disease severity. However, antibiotics in most cases preceded fungicides in antibacterial action.

In almost all cases, disease severity decreased with increasing the concentration of the compound.

Data in Table 18 also show that the compounds varied as regards the best time of application. With both pathogens, contact fungicides (i.e., Dithane Z-78 and Copper oxychloride) gave best results when sprayed 3 days before inoculation. Both antibiotics were most effective against isolate I when applied 3 days before inoculation, but against isolate VII when used one day after inoculation .

Lastly, the antibiotic oxytetracycline chloride was the most effective compound used; in 1974 it completely inhibited infection incited by either isolate when sprayed at 75 ppm 3 days before, or 1 day after, inoculation (Table 18) and in 1975 it either strongly or completely inhibited infection at the mentioned concentration and times of application (Table 19).

D I S C U S S I O N

The present work was conducted to identify the causal organism(s) of a severe head soft rot disease of Kahira Hagin cabbage plants and to obtain necessary informations on the pathogen(s).

Ten isolates of motile, rod-shaped, Gram negative bacteria were obtained from 10 different field-grown cabbage plants. All these isolates were pathogenic to cabbage plants and were reisolated from artificially infected plants. Artificial inoculation of cabbage heads revealed certain differences as regards the severity and type of symptoms induced by different isolates. According to these differences, the isolates could be classified into the following three groups :

1) Mild isolates : isolates I and II showed the mildest symptoms in comparison with those incited by the members of the other 2 groups. Symptoms appeared as water-soaked areas, followed by light brown discolouration, changing to dark brown at the borders, of the affected areas .

2) Moderate isolates : this group involved isolates III, IV, V and VI. Infection with any member of this group was character-

ized by the appearance of water-soaked areas, followed by light brown discolouration of the affected areas .

3) Severe isolates : this group included isolates VII, VIII, IX and X. Infection with any of them appeared as rapidly increasing water-soaked areas, which colour changed to greenish violet then to blackish violet. In addition, small necrotic spots appeared on the midrib, and the veins and veinlets became necrotic .

Morphological, cultural, nutritional and biochemical behaviour of the aforementioned isolates showed that the isolates of each group have similar characters .

Bergey's Manuals of Determinative Bacteriology (1957 and 1974) were consulted for identification of isolates I, III and VII, i.e. the representatives of the three groups of bacteria under investigation .

Table 20 lists most of the examined characteristics of isolates I and III in comparison with those reported for Erwinia carotovora in the 7th edition (1957) and for Erwinia carotovora var. carotovora in the 8th edition (1974) of Bergey's Manual of Determinative Bacteriology. Regardless of some slight

Table 20 :- Morphological, biochemical, physiological and cultural characters of the bacterial isolates I and III in comparison with those reported for Erwinia carotovora var.carotovora

Character	Isolate I	Isolate III	E.carotovora
Shape of cells	Short rods	Short rods	
Size	0.6-0.7 x 0.8-0.9 µ	0.5 x 0.8 - 0.95 µ	0.7 x 1.2 µ
Motility	+	+	+
Pigmentation	-	-	-
Flagellation	2-6 peritrichous	2-6 peritrichous	1-6 peritrichous
Gram reaction	-	-	-
Mucoid growth	+	Faint	d
Spores	-	-	-
Catalase activity	+	+	+
Anaerobic growth	+	+	+
V.P. test	+	+	- or
M.R. test	-	-	+ vary
Starch hydrolysis	-	-	-
Cellulose decomposition	-	-	-
Action on litmus milk	Acid	Acid	Coagulation
Nitrate reduction	+	+	+
Gelatin liquefaction	+	+	+
H_2S production	+	+	+ (from cysteine)
Indol formation	-	-	-
Optimum growth temp.	30	30	27 - 30 °C
Maximum growth temp.	35 - 40	35	37 - 40 °C
Pectate degradation	+	+	+

Table 20 .- Continued

Acid production from organic compounds :			
Arabinose	+	+	+
Xylose	+	+	+
Glucose	+	+	+
Fructose	+	+	+
Galactose	+	+	+
Lactose	+	+	+
Sucrose	+	+	+
Maltose	+	+	+
Dextrin	-	-	-
Starch	-	-	-
Glycerol	+	Weak	(-)
Dulcitol	-	+	(-)
Mannitol	+	+	+
Sorbitol	+	+	+
Ethanol	Weak	-	+
Salicin	+	+	+
Aesculin	+	+	+

+ = positive reaction; - = negative reaction; (-) 20 % or less strains postive; d = 21 - 79 % of strains positive .

differences in certain characteristics, it is clear that both isolates conform with E. carotovora var. carotovora. Therefore, it is believed that isolate I represents a mild form, and isolate III a more severe form of the aforenamed phytopathogenic bacterial organism.

It is to be mentioned here that Rudakov et al.(1950); Muller (1953); Sabet (1954); Del Prado (1959); Oshima and Dickens (1968); Gorlenko (1970); and Chakravarti and Hegd (1972) reported E. carotovora as the causal organism of the soft rot of cabbage .

Isolate VII was found to have the following main properties: strait rods, (0.4 - 0.5 x 1.4 - 2.2 μ). Motile by polar flagella (1 to 3). Gram negative. Strictly aerobic. Produced diffusible fluorescent pigment (green changing to brown). These characteristics obviously show that it is a species of Pseudomonas. Although the strain under investigation cannot be assigned to any well characterized species of Pseudomonas, yet it has many points in common with P. polycolor . These are shown in Table 21.

It is worth mentioning here that in Bergey's Manual of Determinative Bacteriology (1957 and 1974) P. polycolor is reported as animal pathogen (wound, burn and urinary tract

Table 21 .- Certain characteristics of isolate VII in comparison with those reported for P. polycolor

Character	Isolate VII	P. polycolor
Shape of cells	Rods	Rods
Size	0.4-0.5 x 1.4-2.2 µ	0.5-0.8 x 1.5-3.3 µ
Motility	+	+
Pigmentation	+	+
Flagellation	1 - 3 polar	1 - 2 polar, or more
Chains (short)	+	+
Gram reaction	-	-
Capsule	-	-
Catalase activity	+	+
Anaerobic growth	-	-
Starch hydrolysis	-	-
Action on litmus milk	Alkaline	Alkaline
Lipolytic action	+	Weak
Production of ammonia from nitrate	+	+
Gelatin liquefaction	+	+
H_2S production	-	-
Indol formation	-	-
Optimum growth temperature	30 - 35	ca. 37 °C
Growth at 40 °C	+	+
Alkali from citrate	+	+

Table 21 - Continued

Utilization of organic compounds :		
Arabinose	+	+
Xylose	+	+
Glucose	+	+
Fructose	+	+
Galactose	+	+
Lactose	-	-
Sucrose	-	-
Maltose	-	-
Glycerol	Weak	+
Mannitol	Weak	+
Salicin	-	-

+ positive reaction - negative reaction

infections) and occasionally pathogenic for plants (isolated from spots of tobacco).

P. maculicola and P. cichorii were reported as pathogens affecting cabbage heads (Sumner, 1972), but great differences between these organisms and that under consideration do exist and need not be discussed here .

It will be remembered that most of the experimental work was made with isolates I, III (i.e. strains of E. carotovora) and VII (a suspected strain of P. polycolor).

As regards the host range of these strains, all of them could induce rot symptoms, but of varying severity, in the following hosts : Zea mays L. var Amricani badry, Lactuca sativa L. var. longifolia, Lycopersicon esculentum Mill. var. Pritchard, Solanum tuberosum L. var. Alpha, S. melongena L. var. Black Balady, Cucurbita pepo L. var. Eskandarani, Raphanus sativus L. var Balady and Daucus carota L. var. sativa .

The strain of P. polycolor appeared to be of wider host range in comparison with the two strains (I and III) of E. carotovora; it infected 11 plant species out of 12 tested ones. This isolate infected calla and cauliflower whereas both isolates of Erwinia were not able to parasitize these plant species.

Isolate III was unable to infect onion whereas isolate I infected its bulbs only, and isolate VII its tubular green leaves only. Canna plants appeared to be immune to all tested bacteria .

In general, host range studies showed that isolates vary independently as regards severity of symptoms they induced on different hosts. For example, isolate VII induced the most severe rot symptoms in lettuce, whereas in potato and squash such symptoms were incited by isolate III .

Clear hypersensitive reaction appeared 24 hrs after injecting White Burley tobacco leaves (in the mesophyll) with the Pseudomonas isolate (i.e. isolate VII) only, whereas no symptoms appeared in case of inoculation with the two isolates of Erwinia . Klement et al.(1964) found that pseudomonads, pathogenic to plants other than the tobacco, induced hypersensitive reaction when introduced into intracellular spaces of tobacco leaf, whereas P. tabaci (pathogenic to tobacco) did not initiate such effect .

It was found that the head (leaves) of Kahira Hagin cabbage was the main route of infection with the strain of

P. polycolor (isolate VII). Similar results were obtained with other head-rot pathogens of cabbage or Chinese cabbage (Rudakov et al., 1950; Togashi and Sakamoto, 1968; 1969; 1970) .

When the three bacterial isolates were tested, on nutrient glucose agar, against each other for possible antagonistic action, only isolate VII (Pseudomonas) appeared to have inhibitory effect against both other ones .

When studying the interference between isolates in greenhouse tests, it appeared that simultaneous inoculation with any of the Erwinia isolates and the Pseudomonas strain resulted in a reduction of bacterial rot symptoms as compared to inoculation with any of the three involved isolates when tested alone. This indicates that in mixtures the isolates are not synergistic but slightly antagonistic .

Scherff (1973) inhibited the bacterial blight of soybean caused by Pseudomonas glycinea by mixing it with yellow bacterium (YB - 3), at 1 : 9 ratio, before inoculation into soybean leaves .

Under the conditions of the present work, the Pseudomonas isolate was transmitted by a biting insect (cotton leaf worm)

but not by a sucking insect (cabbage aphid). This could be explained assuming that large insects would carry more inoculum and their activity in scratching the leaves would imply greater effectiveness at introducing the bacteria into the head tissues.

Bacterial growth on different media (solid and liquid) and utilization of different organic compounds are adequately described .

Generally, good growth of all isolates was obtained on nutrient-glucose agar, nutrient-glucose agar containing 0.3 % yeast extract, glucose asparagine agar, bouillon glucose, [illegible] (particularly if supplemented with 0.3 % yeast extract), and glucose asparagine solution.

The minimum, optimum, and maximum temperatures for growth of the tested bacteria were : 5 - 10, 30, 35 - 40 °C for isolates of group 1 (Erwinia); < 5, 30, 35 °C for isolates of group 2 (Erwinia); and 15 - 20, 30 - 35, >40 °C for isolates of group 3 (Pseudomonas). The thermal death of all Erwinia isolates was found to be 55 °C, whereas that of Pseudomonas isolates was 57 °C. The optimum pH value for growth of the Erwinia isolates was 6.8 - 7.8, but for the tested Pseudomonas isolate (No. VII) it was 6.2 .

The enzymatic-activity study revealed that all tested isolates of Erwinia and Pseudomonas (i.e., isolates I, III, and VII) were positive for pectolytic enzymes (liquefaction of pectate gel and production of PME, PG, and PP) and for catalase production. With concern to B-glucosidase, the Erwinia isolates were the only ones which showed activity .

Some attempts have been made to correlate virulence of phytopathogenic bacteria witt their enzymatic activities (Sabet and Dawson, 1951; Sabet, 1952; Smith, 1955, Dye, 1960; Walton and Cappellini, 1962; Harborne, 1964; Hildbrand and Schroth, 1964; Rudolph and Stahmann, 1964; Digate, 1971; Abo-El-Dahab and El-Goorani, 1972; Zalewski and Sequeira, 1973; Shihata, 1974; and El-Sadek, 1967) .

The Pseudomonas isolate, which, in comparison with the Erwinia isolates, caused the most severe symptoms in pathogenicity test appeared to be the most active isolate in liquifying pectate gel and in breaking down of pectin. In this respect, Sabet (1952) reported that certain species of both genera liquified pectate gel and that liquefaction of pectate gel could be considerable as a creterion on which to

distinguish phytopathogenic from nonpathogenic organisms .

Generally, the present work shows that no direct relationslip exist between PP activity and PME,PG or liquefaction of a pectate gel. This comes in close agreement with the previously reported results on pectolytic activity in Xanthomonas (Dye, 1960) .

Concerning B-glucosidase activity it was found that both Erwinia isolates secreted the enzyme, whereas the Pseudomonas isolate did not. Such result partially supports those of Shihata (1974) who found that less virulent strains of Streptomyces scabies was more active in B-glucosidase activity. Shihata concluded that B-glucosidase activity could play a role in virulence by means of releasing plant phenols (which, as cited by him after Harborne (1964), occur in plants as glucosides or free compounds) and thus rendering the host tissue more toxic to the invader .

Generally, the auther is inclined to support the conclusion made by El-Sadek (1976) that no generalization may yet be adopted as far as correlation between virulence of phytopathogenic bacteria and their enzymatic activities is concerned .

The toxicity of different phenolic compounds (i.e., pyrocatechol, resorcinol, hydroquinone, p-benzoquinoe) to the bacterial isolates was tested under laboratory conditions . The most active compound was found to be p-benzoquinone, followed by hydroquinone and then pyrocatechol. p-Benzoquinone inhibited the growth at all three tested isolates at 125 ppm, whereas hydroquinone and pyrocatechol suppressed the growth of the Pseudomonas isolate at 125 ppm and of both Erwinia isolates at 250 ppm .

Previous workers found that phenols and, particularly, quinones were highly active against the bacteria they tested (Schaal and Johnson, 1955; Darwish, 1966; Shihata, 1974; and El-Sadek, 1976) .

El-Sadek (1976) found that p-benzoquinone was the most active phenolic compound among those he tested against a member of the Erwinias .

The present study showed that the Pseudomonas isolate was more sensitive to the tested compounds than the Erwinia isolates .

The susceptibility of 2 Erwinia (no. I and III) and 1 Pseudomonas (no. VII) to some antibiotics and fungicides was investigated in vitro and in vivo .

Six fungicides (i.e., Dithane Z-78, Copper oxychloride, Terraclor, Benlate, Bavistin, and Topsin) and 2 antibiotics (Oxytetracycline chloride and Neomycin sulphate) were tested in vitro. It was found that Oxytetracycline and Neomycin were more effective in inhibiting bacterial growth than any of the tested fungicides. Dithane Z-78 followed by Copper oxychloride came next to antibiotics in the antibacterial action .

Oxytetracycline and Neomycin have been previously reported as being highly active against other soft-rot inducing Erwinias (Morgan and Goodman, 1955; Bel'tykova et al., 1966; and Rushdi et al., 1972) and against some phytopathogenic Pseudomons (Katznelson and Sutton, 1951; Shewan, et al., 1954; Morgan and Goodman, 1955; Knosel and Thill, 1966; Knosel, 1967; and Keil and Weaver, 1970) .

As regards fungicides, Dithane Z-78 was reported to be ineffective when tested in medium against E. carotovora at low concentration (El-Helaly et al., 1969); however, at relatively higher concentration (1000 ppm) this fungicide completely in-

hibited growth of E. aroideae, E. carotovora, and E. atroseptica (Rushdi et al., 1972) .

The antibiotics Oxytetracycline and Neomycin, and the contact fungicides Dithane Z-78 and Cu oxychloride (which all and in the order named proved to be highly toxic to the bacteria in vitro) were selected for trials to control the head soft rot of B. oleraceae L. var capitata cultivar Kahira Hagin plants incited by isolate I (Erwinia carotovora) and isolate VII (Pseudomonas polycolor).

Field-grown Kahira Hagin cabbage plants were given only one spray with a suspension (or solution) of the tested compound. Tests included three different times applications (3 or 7 days before inoculation, or one day after inoculation) and three different levels of each compound .

All antobiotics and fungicides tested in this experiment were effective in decreasing disease severity. However, antibiotics in most cases preceeded fungicides in antibacterial action. In most cases, disease severity decreased with increasing the concentration of the compound .

The compounds varied as regards the best time of application. With both pathogens, fungicides gave best results when sprayed 3 days before inoculation. Both antibiotics were most effective against the Erwinia isolate when applied 3 days before inoculation, but against the Pseudomonas isolate when used one day after inoculation .

The antibiotic oxytetracycline chloride was the most effective compound used; in 1974 it completely inhibited infection incited by either isolates when sprayed at 75 ppm 3 days before, or 1 day after, inoculation and in 1975 it either strongly or completely inhibited infection at the mentioned concentration and times of application .

S U M M A R Y

A severe head rot disease of Brassica oleraceae L. var.capitata cultivar Kahira Hagin was encountered in the farms of Faculty of Agriculture, El-Minya University and Secondary School of Agriculture at El-Minya. The purpose of this investigation was to identify, to study the properties of, and to find some control measures against, the pathogenic organism (s) .

Results could be summarized in the folloings :

1 - Ten bacteria were isolated from ten naturally infected cabbage plants. According to morphological, physiological and pathological characters they could be classified into 3 groups. Isolates of group 1 (I and II) and group 2 (III to VI) appeared to be representatives of the genus Erwinia, whereas those of group 3 (VII to X) of the genus Pseudomonas .

2 - Isolates of groups 1 and 2 are non-sporeforming, single straight rods measuring 0.6 - 0.7 x 0.8 - 0.9 µ (group 1) and 0.5 - 0.8 x 0.95 µ (group 2), motile by 2 - 6 peritrichous flagella, surrounded by a sline layer, Gran -

negative. Not lipolytic. Do not hydrolyze starch. Reduce nitrate to nitrite; indol negative. Produce acid and gas from glucose, frutose, and galactose. They are facultative aerobes. Not pigmented. Produce H_2S .

The Pseudomonas isolates are non-sporeforming, single straight rods, sometimes in pairs or short chains, measuring 0.4 - 0.5 x 1.4 - 2.2 µ, motile by 1 - 3 polar flagella, non-capsulated, Gram-negative. Not lipolytic, do not hydrolyze starch. Reduce nitrate to ammonia, indol negative. Produce acid but no gas from glucose, fructose and galactose. They are strictly aerobic and produce fluorescent green pigment. Do not produce H_2S .

The minimum, optimum, and maximum temperatures for growth of the tested bacteria are : 5 - 10, 30, 35 - 40°C for isolates of group 1 (Erwinia); < 5, 30, 35 °C for isolates of group 2 (Erwinia); and 15 - 20, 30 - 35, > 40 °C for isolates of group 3 (Pseudomonas). The thermal death point of all Erwinia isolates is 55 °C whereas that of Pseudomonas isolates is 57 °C. The optimum pH for growth of the Erwinia isolates (I and III) is 6.8 - 7.8, but for the tested Pseudomonas isolate (VII) it is 6.2 .

Good growth of all isolates was obtained on nutrient -glucose agar, nutrient - glucose agar containing 0.3 % yeast extract, glucose asparagine agar, Bouillon glucose (particularly if supplemented with 0.3 % yeast extract), and glucose asparagine solution. They utilized different sugars, alcohols, as well as citrate and tartarate .

Other characteristics are also reported .

The organisms do not decompose cellulose, but rot potato slices. They cause soft rot and necrosis in heads of infected plants .

3 - Morphological and physiological characters revealed that isolates of group 1 and 2 conform with Erwinia carotovora var. carotovora, whereas those of group 3 with Pseudomonas polycolor . Differences between isolates of group 1 and group 2 existed mainly in virulence and slightly in physiological characters .

4 - Artificial inoculation of cabbage heads revealed certain differences as regards the severity and type of rot symptoms incited by different isolates. According to these differences isolates could be classified to mild (group 1), moderate (group 2) and severe (group 3) ones. Rot symptoms

were associated with light brown discolouration (groups 1 and 2) or with blackish violet discolouration plus tissue necrosis (group 3) .

5 - Host range study revealed that the tested isolates (I, III and VII) could produce rot symptoms, but varying severity, in the following hosts : maize, lettuce, tomato, potato, eggplant, squash, raddish, and carrot. The strain of P. polycolor (no. VII) appeared to be of wider host range in comparison with the 2 strains (I and III) of E.carotovora. It infected calla and cauliflower whereas both isolates of Erwinia did not .

6 - Clear hypersensitive reaction appeared 24 hours after injecting White Burley tobacco leaves (in the mesophyll) with the Pseudomonas isolate (i.e., isolate VII) only, whereas no symptoms appeared in case of inoculation with the 2 isolates of Erwinia .

7 - It was found that the head (leaves) of Kahira Hagin cabbage was the main route of infection with the strain of P. polycolor (isolate VII) .

8 - When the bacterial isolates I, III, and VII were tested, on nutrient-glucose agar, against each other for possible antagonistic action, only isolate VII (Pseudomonas) appeared to have inhibitory effect against both other ones.

When studying the interference between isolates in greenhouse tests, it appeared that simultaneous inoculation with any of the Erwinia isolates and the Pseudomonas strain resulted in a reduction of bacterial rot symptoms as compared to inoculation with any of the three involved isolates when tested alone.

9 - Under the conditions of the present work, the Pseudomonas isolate was transmitted among cabbage plants by the cotton leaf worm but not by cabbage aphid .

10 - Enzymatic activities study revealed that all tested isolates of Erwinia and Pseudomonas (i.e., isolates I, III and VII) were positive for pectolytic enzymes (liquefaction of pectate gel and production of PME, PG, and PP) and for catalase production. With concern to B-glucosidase, the Erwinia isolates were the only ones which showed activity.

11 - Tests on toxicity of some phenolic compounds (i.e., pyrocatechol, resorcinol, hydroquinone, and p-benzoquinone) to the bacterial isolates I, III and VII revealed that the most inhibitory substances were:p-benzoquinone followed by hydroquinone and then pyrocatechol. p-benzoquinone inhibited the growth of all 3 tested isolates at 125 ppm, whereas hydroquinone and pyrocatechol suppressed the growth of the Pseudomonas isolate at 125 ppm and of both Erwinia isolates at 250 ppm .

12 - Experiments on the in vitro and in vivo effects of 2 antibiotics and 6 fungicides against the bacterial isolates I, III and VII showed that :

i - Oxytetracycline chloride and Neomycin sulphate were more effective in inhibiting bacterial growth than any of the tesed fungicides (i.e., Dithane Z-78, Copper oxychloride, Terraclor, Benlate, Bavistin,and Topsin). Oxytetracycline chloride caused complete inhibition of the Erwinia isolates at the lowest concentration used (10 ppm) and of the Pseudomonas isolate at 25 ppm, whereas Neomycin sulphate incited such effect to the former isolates at 25 ppm and to the latter one at 50 ppm. The most effective fungicides (Dithane Z-78 and Copper oxychloride) showed such effect