# CHEMICAL AND BIOLOGICAL CONTROL OF SOME PHYTOPATHOGENIC BACTERIA



BY

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# INTRODUCTION

The use of chemicals in controlling plant diseases was and still directed towards those caused by fungous pathogens. Several chemicals were manufactured for this purpose. However, there are reports concerning the effect of certain fungicides in controlling bacterial diseases. (Dye, 1954; Heuberger et al., 1956; Line & Eide, 1960 and Munnecke & Ferguson , 1960) .

On the other hand, the side effects of herbicides on phytopathogenic agents have been noted. The use of herbicides is becoming now a general practice in many countries, and their use increased dramatically from 78 million kg to 147 million kg annually from 1961 to 1972 (Altman & Campbell, 1977). A wide variety of these herbicides are incorporated annually into the soil. It is established that herbicides affect the soil microflora to a certain extent (Bollen, 1961). Thereby soil born phytopathogenic bacteria which spend a part of their life cycle in the soil could also be affected to various degrees. It is important , therefore, to determine the extent to which these herbicides can affect the development of diseases caused by soil born phytopathogenic bacteria.

Because of the environmental hazards of pesticides, ecologists and public health authorities are becoming increasingly aware of the increasing concentrations of toxic

chemicals in nature's food chains. Parallel to the environmentalist's opinion, plant pathologists are becoming aware that upsetting the biological balance by toxic chemicals can lead to severe outbreak of diseases as well as the appearance of new diseases. Moreover, disease control by chemical application is temporary in effect, hence requires repeated applications. On the other hand biological control when effective, is usually more enduring, with no toxic residue in nature's food chains, safe for application, and more cheap in cost.

Therefore, the present investigation was designed to find out among soil microflora some microorganisms which antagonize with some of soil borne phytopathogenic bacteria and compare their usefulness in controlling bacterial plant diseases with some available widely used fungicides and herbicides.

# REVIEW OF LITERATURE

## I- Chemical control:

### 1- Fungicides :

It has been suggested by many investigators that some of fungicides might be valuable in the control of certain bacterial diseases. Moreover, promising information have been obtained about the use of fungicides in the control of combined infections of plants with bacteria and fungi.

Fink (1958) showed that rotting of potato seed-pieces caused by Erwinia atroseptica could be controlled in the laboratory in the absence of soil by streptomycin sulfate at 100 ppm, and that mixtures of different fungicides with streptomycin did not have an adverse effect under these conditions. However, none of the treatments gave control in the field .

Crossan (1959) found that dusting or dipping of seed-pieces with Captan-50 effectively checked decay caused by Erwinia carotovora or Fusarium solani. He also reported that Semesan-Bel gave adequate disease control.

Line and Eide (1960), had pointed out that better protection against decay, caused by Fusarium spp. and Erwinia spp., was obtained by dipping the seed pieces of potato in Maneb (1 lb/10 gal. water), than in Agrimycin or in a mixture of the two.

Robinson et al., (1960) studied the chemical control of black-leg, dry-rot, and Verticillium-wilt of potato. They found that the black-leg of potato could be controlled by $HgCl_2$, Agristrep, and Semesan-Bel.

El-Helaly et al. (1963) studied the bactericidal or bacteriostatic effect of different concentrations of the following phosphorus compounds on local isolate of Agrobacterium tumefaciens. Malathion, Ekatin, Delnaw, Dipterex, Chlorothion, Parathion, Thimet, and Metaisocystox. The bactericidal effect of Streptomycin, Oxytetracycline and Agremycin also was studied. Most of the phosphorus compounds were ineffective bactericides at the minimal inhibitory concentration ranged from 200 to more than 400 ppm. The only phosphorus compound that showed promising bactericidal effect, comparable to the antibiotics was Chlorothion.

El-Kazzaz (1966) studied the effect of available fungicides on certain phytopathogenic bacteria including A. tumefaciens, E. carotovora, and Pseudomonas solanacearum. He found that Nabam, Dithane-$M_{22}$, and Dithane-$M_{45}$ were the only fungicides among these tested that exerted antibacterial property. He found that Dithane-$M_{45}$ was bacteriostatic to the soil borne bacteria, while Nabam was the only fungicide used that showed bactericidal action. He also

found that relatively higher concentrations of Nabam, Dithane-$M_{22}$, and Dithane-$M_{45}$ were required to inhibit growth of the tested bacteria, when inhabiting the soil, than those required In vitro to exert the same action.

Kapustin (1967) reported that disinfection of store-houses with 5% $CuSO_2$, and treatment of seed material in autumn with either Thiram (1-2 kg/ton) or Cuprosan (2 kg/ton) were useful control measures for tuber rot.

Shneider et al., (1968) reported that the most effective preparations against black-leg were 0.5-1 % Captan and 3% Thiram for pre-planting treatment of tubers.

Kuz'Mina (1969) found that treatment of tubers with 2 % Gronosan and 4% Thiram gave a good control of potato scab (Streptomyces scabies), and did not reduce the incidence of black-leg or Fusarium-wilt.

Rushdi et al. (1972) studied the toxicity of some fungicides and antibiotics to soft rot-inducing Erwinia spp. Results indicated that Terramycin and Streptomycin appeared to be have excellent inhibitory effect. Carbazinc inhibited the growth of the soft rot bacteria at a very low concentration. Ceresan, Orthocide, and T.C.N.A. were inferior in this respect.

Mcintosh (1973) studied the effect of Benomyl on potato common scab caused by S. scabies in the glass-house by growing potato plants in soil treated with 50 ppm of the chemical. He found that Benomyl failed to decrease the incidence of scab.

Logan (1974) mentioned that sufficient control against certain potato diseases could be obtained by tubers treatment with Benomyl and Thiabendazol (TBZ).

Shihata (1974) found that Captan, Ceresan, Carbazinc, Cupravit and Dithane-$Z_{78}$ at 100 ppm completely inhibited growth of all isolates of S. scabies.

Weingartner and Shumaper (1974), reported that treatment of fresh potato seed-pieces with fungicides, alone or in combination with antibiotics, gave a protective effect to the infected seed pieces with bacterial-fungal complex.

Mcintosh (1976) used forty quinones, polyhydroxy benzenes and related compounds for possible control of potato common scab, caused by S. scabies. He found that Chloroneb decreased yield, and some 1,4-naphthaquinones and anthraquinones were almost uneffective.

Abo-El-Dahab et al. (1977) studied the effect of certain chemicals on the In vitro growth of A. tumefaciens and on the gall formation on artificially infected plants . They

found that Benlate at concentrations up to 400 ug/ml proved to be ineffective in inhibiting the In vitro growth of the tested isolates of A. tumefaciens, while Carboxin was effective in inhibiting the growth of only certain isolates of A. tumefaciens at concentration 200-400 µg/ml. Carboxin was moderately effective in reducing the number of galls on caster bean seedlings.

Seif-El-Yazal (1980) studied the control of some potato diseases with antimicrobial agents. He found that the fungicides, Zineb, Quinolate-15 and Previcur-N at high concentrations strongly inhibited E. atroseptica growth and to a lesser extent P. solanacearum. However, the three fungicides used were effective as considerably increased the percentage of healthy plants against black-leg disease.

Gabr (1981) studied the In vitro effect of fungicides, Nimrod and Benomyl on the growth of S. scabies. He found that high concentrations (600 ppm) were required for complete inhibition of S. scabies growth , and these two fungicides had no effect on potato common scab disease incidence in pots.

2- Herbicides :

Voluminous work has been done on the effect of tremendous on the biotic potential of soil,e.g. nematodes (Šàly & Ràgala, 1977) , collembola (Ulber ,1977), mites (Dzuiba, 1971), earthworms (Edwards et al.,1970), fungi (Rudakov & Spiridonov, 1979), and certain physiological groups of bacteria contributing to soil fertility (Yurkevich & Tolkachev, 1972 and Helmeczi, 1977).

Regarding to phytopathogenic microorganisms research has been focussed on fungi (Altman & Campbell, 1977 and Hassan, 1981). Yet , few workers have studied the effect of certain herbicides on phytopathogenic and related bacteria.

Rankow (1971) , found that Pseudomonas sp. developed well in medium where Treflane was the only source of nitrogen.

Carter and Camper (1973), studied the bacterial populations in soil receiving repeated applications of Trifluralin. They found that Pseudomonas-like was the predominant bacteria recovered from soil.

Ulasevich (1978) found that Linuron increased populations of Pseudomonas , Sarcina , and Bacillus spp.

Gabr (1981) studied the In vitro effect of six herbicides Trifluralin, Dinitramine, Fluometuran, Dalapon , Diuron, and Prometryn on the growth of S. scabies . He found that Dinitramine inhibited the growth of S. scabies at a relatively high concentration of 200 ppm . Other herbicides were least effective , higher concentrations (600 ppm) were required for complete growth inhibition.

A report from the Canadian Agricultural Research Institute (1979) indicated that through researchs upon the effect of herbicides on non-target micro-organisms , it was found that resistance to Paraquat by Agrobacterium radiobacter was increased up to 30 000 µl / ml.

II- Biological control:

Numerous investigations have demonstrated the possibility of effective utilization of microorganisms for restricting and suppressing the development of several plant pathogens, particularly, in the case that the usage of chemicals is ineffective.

Goodman and Henry (1947a), reported that a rough strain of B. subtilis, was found to be relatively non-phytotoxic and display marked antagonism towards certain bacteria, i.e., Xanthomonas translucens, Pseudomonas coronofaciens , Erwinia carotovora, and Agrobacterium tumefaciens.

Goodman and Henry (1947b) reported that subtilin an antibiotic substance produced by Bacillus subtilis reduced infection of Xanthomonas translucens , to barley seedlings when they were treated simultaneously or when subtilin was applied 4 to 5 days after the seed had been infested with the pathogen .

Stessel et al. (1953) isolated toximycin from Bacillus subtilis which inhibited growth of phytopathogenic fungi and bacteria including Streptomyces scabies.

Teliz-Ortiz and Burkholder (1960), isolated a strain of Pseudomonas fluorescens antagonistic to P. phaseolicola and many other bacterial pathogens.

Abo-El-Dahab and El-Goorani (1964) showed that growth of Erwinia amylovora was inhibited by culture filterates of Bacillus subtilis grown on sucrose nutrient broth.

Deep and Young (1965) showed that preplanting fungicide treatments increased the incidence of crown gall of cherry seedlings, suggesting the presence of natural microbial competitors.

Drummond and Lea Barg (1965), found that Pseudomonas solanacearum could be controlled by a strain of antagonistic Streptomyces sp. which remained viable in mixture of chemical fertilizers or pure lime applied to the soil.

Weinhold and Bowman (1968), found that in a long-term field experiment a soybean cover-crop and green-manure incorporation prevented the build-up of common scab of potato. When soil from these plots was assayed for organisms antagonistic to Streptomyces scabies , a bacterium identified as Bacillus subtilis was found to be predominant.Laboratory tests showed that S. scabies was sensitive to the antibiotic produced by this bacterium .

Abo-El-Dahab and El-Goorani (1969) studied the antagonism among strains of Pseudomonas solanacearum, on three different agar media with or without organic nitrogen . They found that both virulent and avirulent cells can produce a growth inhibitory principle(s). They added also that the amount of such principle(s) produced by the virulent strain could inhibit growth of its own type,but was not enough to inhibit the avirulent growth. Also , the effect of inhibitory principle(s) was bacteriostatic, since inocula transferred from the inhibitory zones were able to resume growth on glycerol agar.

Kerr (1972), achieved significant biological control of crown-gall when peach seeds were inoculated with the nonpathogenic isolate 84 of Agrobacterium radiobacter var. radiobacter before sowing in natural soil heavily inoculated with the tumour inducing isolate 27 (A. radiobacter var. tumefaciens), while dusting seed with Thiram (3.1 g/ kg seed) did not significantly reduce disease incidence.

Nair and Fahy (1972) isolated three bacteria antagonistic to Pseudomonas tolasii the causal agent of brown blotch of the cultivated mushroom, from soil and peat . These were a non-fluorescent Pseudomonas sp. (closest to P. multivorans) from soil, and strains of P. fluorescens

and Enterobacter aerogenes from peat. They found that when the antagonists and the pathogen were added in the ratio of 8 x $10^7$ : $10^6$ cells/ml to unsterilized peat and applied to mushroom trays, infection by pathogen was effectively controlled.

New and Kerr (1972) achieved 100% control by dipping roots of young peach seedlings in a cell suspension of Agrobacterium radiobacter var. radiobacter before planting in soil heavily infested with an isolate of the pathogen A. radiobacter var. tumefaciens.

Rangaswami (1972), isolated an antibiotic produced by a Streptomyces isolate, had antibacterial activity against Pseudomonas solanacearum .

Mall (1973), studied the microbial antagonism in the rhizosphere-soils of potato varieties differentially susceptible to black-scurf and wilt at different stages of growth. It was concluded that the antagonists were always found in higher frequency in rhizosphere as compared with check soil.

Sidibe (1973), stated that the composition of mycoflora antagonistic to Streptomyces scabies depended on whether potatoes were grown in mono-culture or in a crop rotation. The degree of antagonism by Aspergillus,

Fusarium, Penicillium and Trichoderma spp. varied sharply. He also added that some of the fungi were antagonistic to one another limiting the possibility of using them to control S. scabies.

Haty et al. (1974), studied the biological control of crown-gall by means of seed and root inoculation with non-pathogenic strain of Agrobacterium radiobacter. They reported a significant disease control by this method and commented that this method of biological control is widely practised by commercial growers in South Australia.

Mohamed (1974) studied the antagonism of Bacillus subtilis strains against certain plant diseases causal organisms. He found that Streptomyces scabies isolates were sensitive to B. subtilis antibiotic(s),while growth of Agrobacterium tumefaciens and Erwinia carotovora was not affected. He added that addition of B. subtilis bacterial suspension or culture filtrate failed to control potato scab disease.

Farag (1976), found that sporeformers antagonistic to Pseudomonas solanacearum were identified as strains of Bacillus subtilis, B. licheniformis, and B. cereus. He also studied the relation of the wilt bacterium to other potato pathogens, and the results showed that P.

solanacearum did not inhibit the growth of Erwinia carotovora, E. atroseptica, Fusarium solani, and F. oxysporum.

Burr et al. (1978), found that significant increases in growth and yield of potato plants were achieved by treating seed pieces with suspensions of two Pseudomonas spp. prior to planting. The pseudomonads were isolated from the surface of potato, and were identified as strains of P. fluorescens and P. putida. The isolates exhibited In vitro antibiosis against Erwinia carotovora var.carotovora . This lead them to say that the mechanism by which these isolates enhance plant growth and tuber yield may be associated with changes in the composition of rhizosphere bacterial flora .

Süle (1978) obtained a good biological control of crown-gall with a peat cultured antagonist , where strain 84 of Agrobacterium radiobacter may survive at least six months in peat culture. In field experiments, applications of the peat culture effectively controlled crown-gall.

Cooksey and Moore (1980) studied the biological control of crown gall with fungal and bacterial antagonists. They found that pathogenic Agrobacterium

strains were In vitro inhibited by different fungi and bacteria that were isolated from nursery soils in Oregon and Washington. In field tests, isolates of Penicillium, Aspergillus, Bacillus, Pseudomonas, and Agrobacterium radiobacter reduced the incidence of galling on mazzard cherry seedling.

Mahmoud et al. (1981a) studied the correlation between rhizosphere microflora and susceptibility to common scab of potatoes. They found that potato varieties greatly varied in their susceptibility to five Streptomyces scabies pathogenic isolates. Densities of different groups of microorganisms highly affected by potato varieties. Actinomycetes, spore-formers and fungi were higher in the rhizosphere of Alpha var. than other varieties inoculated with virulent S. scabies isolates. King-Edward var. showed, in unoculated soil, higher densities of total microbial flora, spore-formers and fungi. They mentioned that these two varieties, Alpha and King Edward , showed less susceptibility to S, scabies.

Mahmoud et al. (1981b), in a preliminary study of antagonistic microorganisms to S. scabies in the rhizosphere of some potato varieties, found that the rhizosphere of Alpha variety contained high percentages of antagonistic

spore-formers , most of them found to be Bacillus subtilis and B. cereus, while the rhizosphere of King Edward variety gave the highest percentage of antagonistic actinomycetes, most of them belonged to Streptomyces albus. They proved that fungal isolates did not show any antagonism to S. scabies.

## MATERIALS AND METHODS

The phytopathogenic bacteria used in this investigation, were provided by the phytobacteriology sections of the Plant Pathology Departments, Faculties of Agric., Alex. Univ., and Tanta Univ., Egypt. The tested phytopathogenic bacteria were :

1. Agrobacterium tumefaciens (Smith and Townsend) Conn, the causal agent of crown-gall disease.

2. Erwinia atrospetica (Van Hall) Jennison, the causal agent of black-leg disease in potato.

3. Erwinia carotovora (L.R. Jones) Holand, the causal agent of soft-rot disease.

4. Streptomyces scabies (Thaxter)Waksman and Henrici, the causal agent of common-scab disease in potato.

After ensuring their cultural, morphological, and pathological characteristics, the aforementioned pathogenic bacteria were subjected to the tests described below.

### I- In vitro experiments:

#### 1- Effect of some fungicides and herbicides on the growth of phytopathogenic bacteria:

(A) Sensitivity of phytopathogenic bacteria towards fungicides and herbicides:

Ten widely used fungicides and (preemergence) herbicides were tested to find out their direct toxic effect upon phytopathogenic bacteria. Chemical formules, percentage of the active ingredient, and name of manufacturers of the tested chemicals are presented in table (1). Streptomycin, the active antibiotic against many bacterial plant pathogens, was used for comparison.

The disc diffusion method (Thornberry, 1950) was used for evaluating the inhibitory effect of the used chemicals. In this method, filter paper disc (10 mm diam.), impregnated with 0.05 ml portion of the used concentrations, was placed on the surface of glycerol nutrient agar medium, subsequently inoculated with the bacterial (48 hr. old) inocula. The degree of the inhibitory action was estimated by measuring the diameter of the inhibition zones surrounding the discs after 48 hr. incubation period at 28-30°C.

(B) The least bactericidal concentration (L.C.C.):

The least bactericidal concentration of the most effective chemicals for each tested phytopathogenic bacteria was determined as follows :

Table (1): Commercial name, chemical name, active ingredient, and manufacturer of the chemicals .

| Commercial name | Chemical name | Active ingred-ient | Manu-facturer |
|---|---|---|---|
| Fungicides: | | | |
| Benlate (w.p.) | Methyl-2-benzimidazol Carbamat (M.B.C.) | 50% | (Du-Pont de Nemours & Co.Inc.) |
| Homai-80 (w.p.) | Thiophanate Methyl(50%) + Bis (dimethyl thiocarbamyl disul-fide) (30%) | 80% | (Nippon Soda) |
| Rovral (w.p.) | 1-isopropylcarbamoyl-3-(3,5-di-chlorophenyl-hydantion) | 50% | (Rhône-Poulenc) |
| Tecto (w.p.) | 2-(4-thiazolyl)-benzimadazole | 60% | (Merch & Co.Inc.) |
| Vitavax/ Captan (w.p.) | 5-6 Dihydro-Methyl-1,4 Oxathin 3-Carboxanilide (37.5%) + N-trichloromethylthio-4-cyclo-hexen-1,2 dicarboximide (37.5%) | 75% | (Uniroyal) |
| Herbicides: | | | |
| Bladex (w.p.) | 2-(4-chloro-6-ethylamino-s-triazin-2-yl) amino-2-methyl-propionitrile | 80% | (Shell Inter. Chemical Com.) |
| Enide (w.p.) | N,N-dimethyl-2,2-diphenylacet-amide . | 80% | (Upjohn Com.) |
| Eptam (E.C.) | S-ethyl dipropylcarbamothioate | 76.97% | (Stauffer Chemical Com.) |
| Linuron (w.p.) | 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea. | 50% | (Hoechst AG) |
| Sencor (w.p.) | 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5 (4 H)-one. | 70% | (Bayer Leverkusen) |

Poisoned glycerol agar plates containing the fungicide under investigation were prepared using serial concentrations (i.e., 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 100 and 200 ppm of the active ingredient). Each phytopathogenic bacterium was streaked on the surface of agar (4 streaks/plate) . Five plates were used for each treatment. Glycerol agar plates without fungicide were used as control. After incubation at 28-30°C for three days, the absence or presence of bacterial growth was recorded. The absence or presence of viable cells on plates that showed no obvious growth was confirmed by further transmission to glycerol agar or glycerol broth containing no chemical . These aforementioned experiments were carried out twice, and the highest dilution of the chemical that prevented the growth was considered the least bactericidal concentration.

2. Screening of antagonists against the tested phytopathogenic bacteria:

(A) Isolation and purification of antagonists:

Since the tested phytopathogenic bacteria are considered as soil and seed-borne agents, screening of antagonists was carried out using collected soil samples.

Since potatoes are the main host of three phytopathogenic bacteria under investigation, i.e. Erwinia carotovora , Erwinia atroseptica, and Streptomyces scabies , soil samples were collected from fields previously cultivated for several years with potatoes in Kafr El-Sheikh and Dakahlia governorates. For the fourth phytopathogenic bacterium Agrobacterium tumefaciens, screening was made using soil samples taken from orchards of stone-fruits and poms located in El-Qanater El-Khairia, Qalubia governorate.

Five rhizosphere-soil samples were collected from different locations in each field, air dried and mixed . Ten grams were added to 90 ml sterilized water in conical flask (250 ml.). After thoroughly shaking for 10 min. dilution series up to 1 : 1,000,000 were prepared. The three later dilutions were used to inoculate P.D.A.medium plates ; one-tenth ml. portion of soil suspension was spreaded on a plate surface using sterilized Drigalasky glass triangle. Plates were inoculated at room temperature for 1-3 days. After incubation, plates containing separate colonies, were sprayed with turbid suspensions of the tested phytopathogenic bacteria until plates were evenly wetted but not to runoff. Inocula of these bacteria were

prepared from 48 hr. old cultures grown on glycerol broth. After incubation for further 24-48 hr.at 28°C,colonies exhibiting antibiosis were picked up,examined for purity,and stored. Isolates, which proved to be antagonistic against any of the pathogenic bacteria were tested also against the others to select those antagonists which exhibit the broadest inhibitory spectrum against the tested phytopathogenic bacteria.

(B) Estimation of efficiency of the antagonistic isolates:

Two aspects were considered in the efficiency estimation : i) spectrum of the affected phytopathogenic bacteria, and ii) relative power of antibiosis against each of the tested phytopathogenic bacteria.

Isolates, which proved to have inhibitory effect against the tested phytopathogenic bacteria were subjected to standardized test to select those having the highest antagonistic power against all of the tested bacterial pathogens as follows : Glycerol agar medium was poured into petri-dishes (15 ml/dish), after solidification,plates were placed in a 45°C oven with lids partially open for 4 hr . to remove excess surface water. Standardized bacterial growth ( 24 hr. old ) of each isolate was spotted at the periphery of glycerol agar plates (four isolates/dish). After incuba-

tion for 48 hr.at room temperature,diameter of each spotted growth was recorded,and plates were evenly sprayed with cell suspensions of the tested phytopathogenic bacteria as mentioned before in 2(A). Plates were reincubated at 28°C and diameter of the inhibition zone surrounding each isolate was recorded after 48 hr. All the aforementioned experiments were made in three replicates and a relative power of antibiosis of each isolate was estimated through the ratio :

$$(R.P.A.) = \frac{Z}{C}$$

where: (R.P.A.): relative power of antibiosis,
Z : diameter of inhibition zone after 48 hr. from spraying with phytopathogenic bacterium,
C : diameter of spotted antagonistic isolate after 48 hr. incubation.

(C) Identification of antagonistic isolates:

Antagonists were subjected to morphological and standard staining tests. For the most efficient isolates (i.e. $B_1$, $B_{43}$, and $S_{41}$), furthermore cultural morphological,and physiological properties were tested. In these tests the standard methods recommended by the Society of the American Bacteriologists (1957), by Smith et al. (1952), by Pelczar & Reid(1958) and by Király et al. (1974) were followed .

3- Antibiosis between phytopathogenic bacteria:

To determine whether any of the tested phytopathogenic bacteria antagonizes any of the others , similar antibiosis tests were carried out as shown before in 2 (B) .

II- In vivo experiments:

Chemicals and antagonists which proved In vitro to be most effective against the tested phytopathogenic bacteria, were subjected to further study in pot experiments in order to control bacterial diseases. These experiments were carried out in out-doors using sterilized pots filled with loamy soil. Pots were sterilized by soaking in 5 % formalin for 5 min. Soil was spreaded in a thin layer, impergnated with 2% formalin and covered with polyethylene for 2 days. The formalin-treated pots and soil were left for 15 days before planting.

1- Crown-gall disease:

(A) Chemical control:

Sun-flower seedlings (cv. Giza-1) were used as test plants. Seeds were germinated in sterilized 30 cm. pots filled with sterilized sandy loam soil. Transplants (35 days old) were washed free from the adhering soil, wounded by sterile syringe-needle (10 wounds/plant) in the crown region and immersed (for 5 min.) in a solution of the chemical to be tested at different concentrations. After dryness, transplants were immersed (for 5 min.) in a water-washed cell suspension of Agrobacterium tumefaciens containing $10^7$ colony-forming units (cfu)/ml. Treated

transplants were transplanted in sterilized (15 cm.) pots containing sterilized clay loamy soil.

Inoculated transplants receiving no chemical, and others chemically treated but uninoculated , served as check treatments. All the aforementioned treatments were represented with 15 plants.

Four weeks after transplanting, the formed galls were separated from plants of each treatment and weighed . Percentage of tumor inhibition was determined according to (Teufik, 1972) using the following equation :

$$\text{Gall inhibition percentage (G.I.P.)} = 100 - \left(\frac{a}{b} \times 100\right)$$

where: a = the average weight of galls on treatment .

b = the average weight of galls on control.

(B): Biological control:

Inocula of the antagonistic isolates were prepared by growing them on glycerol nutrient broth medium with the aid of magnetic shaking . Washed growth was blended in sterilized water and cell concentrations were adjusted to be $10^6$, $10^7$ and $10^8$ cfu /ml. Sunflower transplants were wounded in the crown region (10 wounds/plant) , immersed (for 5 min.) in the antagonistic suspension to be tested, and allowed to dry. Then, transplants were

immersed (for 5 min.) in a water-washed cell suspension of Agrobacterium tumefaciens containing $10^7$ cfu /ml., and transplanted. Check treatments included transplants inoculated only with antagonistic isolates, and others treated only with the pathogenic bacterium.

Each treatment was represented by 15 plants. Four weeks after transplanting, galls formed were separated from plants, weighed and the effect of each treatment on the incidence of crown-gall disease was determined using the same equation mentioned before in chemical control.

2- Potato black-leg and soft-rot diseases:

(A): Chemical control:

Sterilized (25 cm.) pots, filled with (5 kg / pot) sterilized loamy clay soil, were used. Healthy seed-tubers of potato (cv. Arran Banner) were surface sterilized in 0.1% mercuric chloride solution for 5 min., then washed for 10 min. in sterilized tap water. Standard eye-pieces were removed by a flame-sterilized knife. Seed-pieces were immersed (for 5 min.) in a solution of the chemical to be tested at different concentrations, then seed-pieces were kept up to dryness before inoculating with the bacterial pathogen. Inocula were prepared by growing bacteria on glycerol nutrient broth and washed cells were blended

in sterilized distilled water. Inoculation was carried out by dipping surface-sterilized seed-pieces in a suspension of either Erwinia atroseptica or E. carotovora containing $10^7$ cfu /ml. Then, seed-pieces were planted in pots at depth of 4 cm. (one piece per pot), and irrigated every other day .

Check treatments without chemical application or without inoculation with pathogenic bacteria were also included. For each of the aforementioned treatments , ten replicates were made.

The percentages of healthy plants (in case of blackleg) or of emerged plants (in case of soft-rot) were recorded and the efficacy of the chemical treatment was determined using the following formula (according to Seif El-Yazal, 1980):

$$\frac{\%\text{ healthy(or emergent)plants in treatment} - \%\text{ healthy (or emergent) plants in control}}{\%\text{ healthy (or emergent)plants in treatment}} \times 100$$

(B): Biological control:

Inocula of the antagonistic isolates were prepared as described before, and cell concentrations were adjusted to be $10^6$, $10^7$, and $10^8$ cfu /ml. Seed-pieces were immersed (for 5 min.) in the suspension of the antagonistic isolate

to be tested . Then, seed-pieces were kept to dryness before inoculating with the bacterial pathogen. Preparation of pathogenic inocula, inoculation, planting in pots, and irrigation were carried out in the same manner as described before in chemical control.

Check treatments included: (i) inoculation with the pathogen without application of the antagonistic isolates, and (ii) application of the antagonistic isolates in the absence of the pathogens. For each experimental treatment, ten replicates were made.

The efficacy of each antagonistic isolate on disease incidence was determined as mentioned before in chemical control.

3- Common-scab disease of potatoes:

(A) Chemical control:

Sterilized pots (30 cm.), filled with sterilized sandy loam soil (6 kg/pot) , were used. Chemical to be tested was added to the soil at different concentrations before planting. Healthy standarized seed-tubers of potato (cv. King Edward) were surface sterilized in 0.1% mercuric chloride solution for 5 min., washed for 10 min. in running tap water, and planted at depth of 4 cm. in soil. Soil infestation with Streptomyces scabies was carried out at

planting by adding 200 ml. of washed, water blended, bacterial growth at a concentration of $10^7$ cfu /ml.

Check treatments included pots infested with the pathogen, and others treated with the chemical without infestation. For each treatment, ten replicates were made. At the end of growth season, tubers were harvested and examined for the occurence of scab lesions. Severity of the disease in each treatment was determined using following disease index described by Shihata(1974).

$$\text{Disease index } \% = \frac{0A + 1B + 2C + 3D + 4E + 5F}{5T} \times 100$$

where:

* Numerical grades:-
  - 0 = no symptoms.
  - 1 = trace-10% of tuber surface is scabed.
  - 2 = 11 - 20% " " " " " .
  - 3 = 21 - 30% " " " " " .
  - 4 = 31 - 40% " " " " " .
  - 5 = more than 40% of tuber surface is scabed.
* A, B, C, D, E, and F are the number of tubers corresponding to the numerical grades, respectively.
* T is the total number of tubers, i.e. $T = A + B + C + D + E + F$ .

(B) Biological control:

Healthy standarized seed-tubers of potato (cv. King-Edward) were surface sterilized, washed, and planted at depth of 4 cm. in sterilized pots 30 cm. (each filled with 6 kg. sandy loam soil). Soil infestation with Streptomyces scabies was carried out immediately after planting by adding washed cell-suspension containing $10^7$ cfu/ml to pots (200 ml/pot). Inoculation with antagonistic isolates was carried out simultaneously, by adding washed cell-suspension of the antagonistic isolate to be tested containing either $10^6$, $10^7$, or $10^8$ cfu /ml (200 ml/pot).

Check treatments included pots infested only with S. scabies and others inoculated only with antagonistic isolates. Each treatment was represented by 10 replicates. At the end of the growth season, tubers were digged out and examined for the occurence of scab lesions. The effect of the antagonistic isolates was determined using the same disease index mentioned before in chemical control.

Statistical analysis of collected data was done as described by (Snedecor, 1956) and mean values were compared by Duncan's multiple range test (1955).

## EXPERIMENTAL RESULTS

### I- In vitro experiments:

#### 1- Effect of some fungicides and herbicides on the growth of the tested phytopathogenic bacteria:

Sensitivity of four phytopathogenic bacteria (*Agrobacterium tumefaciens*, *Erwinia atroseptica* , *E. carotovora*, and *Streptomyces scabies*) towards 5 fungicides and 5 herbicides were studied using the disc diffusion method. Fungicides tested were Benlate, Homai-80, Rovral, Tecto, and Vitavax/Captan. Herbicides tested were Bladex, Enide, Eptam, Linuron, and Sencor. For comparison , the effective antibiotic Streptomycin was also included in this experiment. Furthermore, the least bacterial concentration (LCC) of the effective chemical was also determined.

(A) Sensitivity of phytopathogenic bacteria towards fungicides and herbicides:

Data in Table(2)show the diameter of inhibition zones produced by the tested chemicals at nine levels of concentrations(i.e.1000,500,250,150,100, 50, 25, 12.5, and 6.25 µg/disc). Results indicate that:

(1) Benlate had no direct inhibitory effect upon the growth of any of the tested phytopathogenic bacteria.The same result had been obtained in cases of the fungicide

Table (2): Sensitivity of four phytopathogenic bacteria towards different concentrations of 5 fungicides, 5 herbicides and Streptomycin sulfate using the disc diffusion method.

| Chemicals | Diameter of growth inhibition zones ( in mm. ) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1000 | 500 | 250 | 150 | 100 | 50 | 25 | 12.5 | 6.25 |
| | Agrobacterium tumefaciens | | | | | | | | |
| Fungicides: | | | | | | | | | |
| Benlate | - | - | - | - | - | - | - | - | - |
| Homai-80 | 25 | 23 | 15 | 15 | 15 | 12 | 11 | - | - |
| Rovral | - | - | - | - | - | - | - | - | - |
| Tecto | - | - | - | - | - | - | - | - | - |
| Vitavax/Captan | 17 | 15 | 13 | 12 | 12 | 11 | - | - | - |
| Herbicides: | | | | | | | | | |
| Bladex | - | - | - | - | - | - | - | - | - |
| Enide | - | - | - | - | - | - | - | - | - |
| Eptam | - | - | - | - | - | - | - | - | - |
| Linuron | - | - | - | - | - | - | - | - | - |
| Sencor | - | - | - | - | - | - | - | - | - |
| Streptomycin | 21 | 19 | 14 | 13 | - | - | - | - | - |

| Chemicals | Diameter of growth inhibition zones ( in mm. ) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1000 | 500 | 250 | 150 | 100 | 50 | 25 | 12.5 | 6.25 |
| | Erwinia atroseptica | | | | | | | | |
| Fungicides: | | | | | | | | | |
| Benlate | - | - | - | - | - | - | - | - | - |
| Homai-80 | 36 | 35 | 35 | 34 | 30 | 27 | 24 | 22 | 16 |
| Rovral | - | - | - | - | - | - | - | - | - |
| Tecto | - | - | - | - | - | - | - | - | - |
| Vitavax/Captan | 11 | - | - | - | - | - | - | - | - |
| Herbicides: | | | | | | | | | |
| Bladex | - | - | - | - | - | - | - | - | - |
| Enide | - | - | - | - | - | - | - | - | - |
| Eptam | - | - | - | - | - | - | - | - | - |
| Linuron | - | - | - | - | - | - | - | - | - |
| Sencor | - | - | - | - | - | - | - | - | - |
| Streptomycin | 46 | 45 | 40 | 39 | 38 | 36 | 32 | 29 | 26 |

| Chemicals | Diameter of growth inhibition zones ( in mm. ) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1000 | 500 | 250 | 150 | 100 | 50 | 25 | 12.5 | 6.25 |
| | Erwinia carotovora | | | | | | | | |
| Fungicides: | | | | | | | | | |
| Benlate | - | - | - | - | - | - | - | - | - |
| Homai-80 | 34 | 34 | 34 | 34 | 32 | 27 | 21 | 20 | 17 |
| Rovral | - | - | - | - | - | - | - | - | - |
| Tecto | - | - | - | - | - | - | - | - | - |
| Vitavax/Captan | 12 | 11 | 11 | 11 | 11 | 11 | - | - | - |
| Herbicides: | | | | | | | | | |
| Bladex | - | - | - | - | - | - | - | - | - |
| Enide | - | - | - | - | - | - | - | - | - |
| Eptam | 11 | 11 | 11 | - | - | - | - | - | - |
| Linuron | - | - | - | - | - | - | - | - | - |
| Sencor | - | - | - | - | - | - | - | - | - |
| Streptomycin | 41 | 40 | 37 | 37 | 36 | 34 | 31 | 28 | 26 |

| Chemicals | Diameter of growth inhibition zones ( in mm. ) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1000 | 500 | 250 | 150 | 100 | 50 | 25 | 12.5 | 6.25 |
| | Streptomyces scabies | | | | | | | | |
| Fungicides: | | | | | | | | | |
| Benlate | - | - | - | - | - | - | - | - | - |
| Homai-80 | 44 | 41 | 40 | 39 | 32 | 30 | 25 | 21 | 14 |
| Rovral | 26 | 24 | 23 | 22 | 21 | 17 | - | - | - |
| Tecto | - | - | - | - | - | - | - | - | - |
| Vitavax/Captan | 22 | 21 | 19 | 17 | 16 | 12 | - | - | - |
| Herbicides: | | | | | | | | | |
| Bladex | - | - | - | - | - | - | - | - | - |
| Enide | - | - | - | - | - | - | - | - | - |
| Eptam | - | - | - | - | - | - | - | - | - |
| Linuron | 21 | 19 | 18 | 17 | 14 | 12 | - | - | - |
| Sencor | - | - | - | - | - | - | - | - | - |
| Streptomycin | 57 | 51 | 49 | 48 | 47 | 46 | 45 | 43 | 39 |

* µg active ingredient per disc.

Tecto, and the herbicides Bladex, Enide and Sencor.

(2) Eptam had no direct inhibitory effect against Erwinia atroseptica, Agrobacterium tumefaciens, and Streptomyces scabies, but in the case of Erwinia carotovora Eptam showed negligible inhibitory effect at the highest tested concentrations(250-1000 ug/disc).

(3) Rovral and Linuron were ineffective in all cases, but one exception arised in the case of Streptomyces scabies which showed sensitivity towards Rovral and Linuron recorded as inhibition zones of 26 and 21 mm. (in diam.) respectively at the highest tested concentration (1000 µg/disc).

(4) Vitavax/Captan showed slight inhibitory effect in all cases. Zones of inhibition ranged from 11 mm (in diam.) in the case of Erwinia atroseptica to 22 mm. in the case of Streptomyces scabies at the highest tested concentration (1000 µg/disc).

(5) "Homai-80", compared with other fungicides and herbicides, showed relatively larger zones of inhibition at all used concentrations against all the tested phytopathogenic bacteria. "Homai-80" had direct inhibitory effect even at the least used concentration (6.25 µg/disc) in all cases except in the case of Agrobacterium tumefaciens which tolerated concentrations of Homai-80

up to 12.5 µg/disc. Figs. 1, 2, 3 and 4 show the inhibitory action of Homai-80 against the tested phytopathogenic bacteria.

(6) Streptomycin inhibited the growth of all tested phytopathogenic bacteria at relatively low concentrations. At the least used concentration (6.25 µg/disc), Streptomycin showed larger inhibition zones, except in the case of Agrobacterium tumefaciens which showed tolerance towards streptomycin concentrations up to 100 µg/disc.

(B): The least bactericidal concentration (LCC):

Table (3) shows the least bactericidal concentrations of the most effective chemical (Homai-80) compared with Streptomycin. From data given in table (3) the following could be concluded:

(1) "Homai-80" inhibited the growth of all tested bacteria at markedly low concentrations. The minimal inhibitory concentrations of Homai-80 landed under 1 ppm in the case of Streptomyces scabies , between 20-25 ppm in cases of Erwinia atroseptica and E. carotovora, and between 25-30 ppm in the case of Agrobacterium tumefaciens. Inocula, transferred from inhibited streaks in cases of all tested bacteria, had no viability and could not resume growth on media free from Homai-80, suggesting

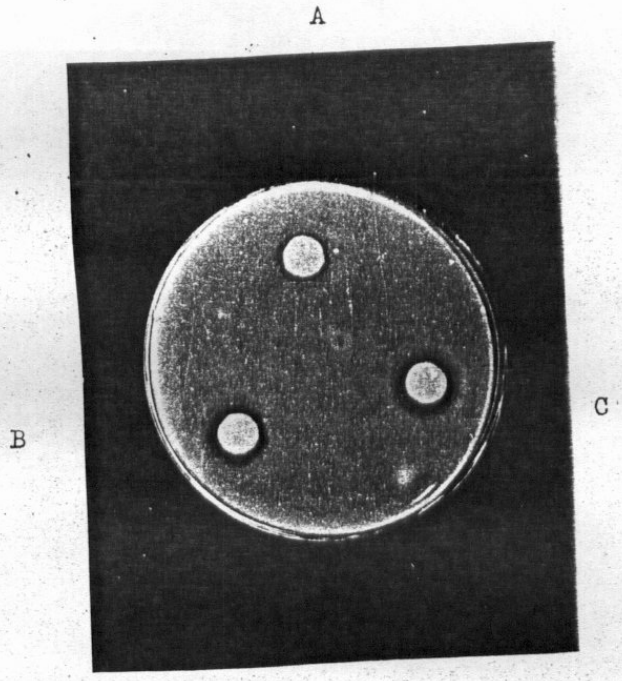



Fig. (1): Effect of Homai-80 on the growth of Agrobacterium tumefaciens using disc diffusion method at different concentrations.

A = 50 µg/disc.
B = 100 µg/disc.
C = 150 µg/disc.

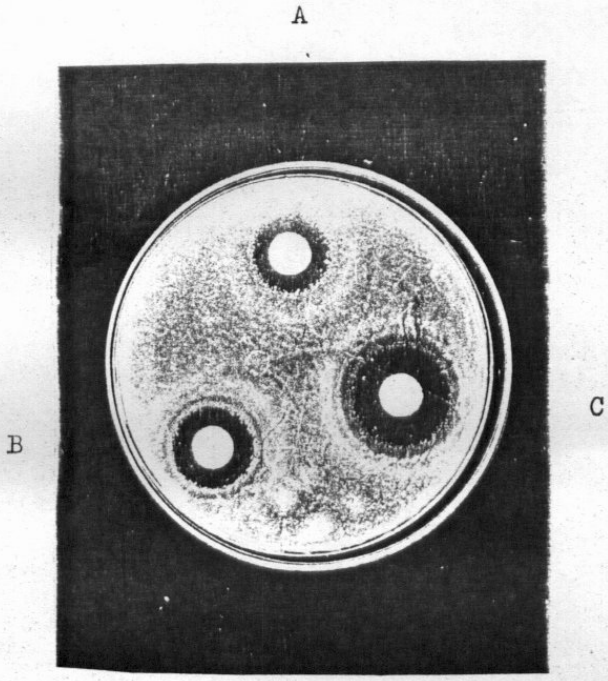



Fig. (2): Effect of Homai-80 on the growth of Erwinia atroseptica using disc diffusion method at different concentrations.

A = 50 µg/disc.
B = 100 µg/disc.
C = 150 µg/disc.

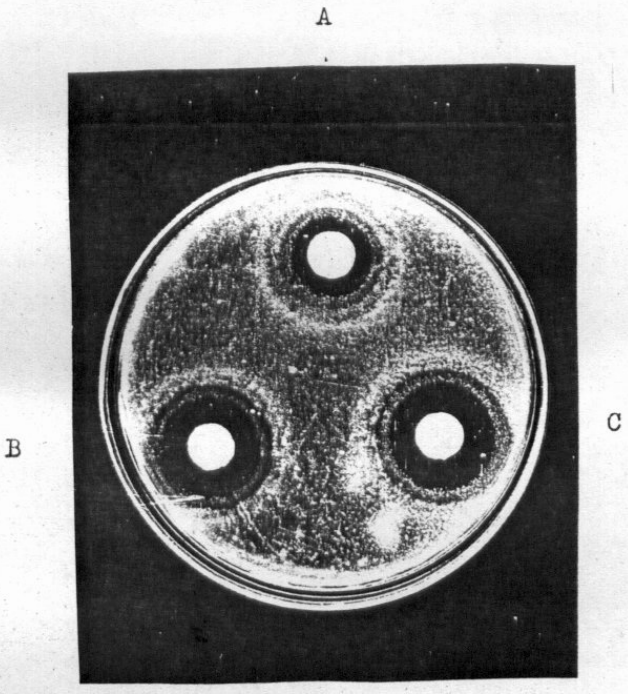



Fig. (3): Effect of Homai-80 on the growth of Erwinia carotovora using disc diffusion method at different concentrations.

A = 50 µg/disc.
B = 100 µg/disc.
C = 150 µg/disc.

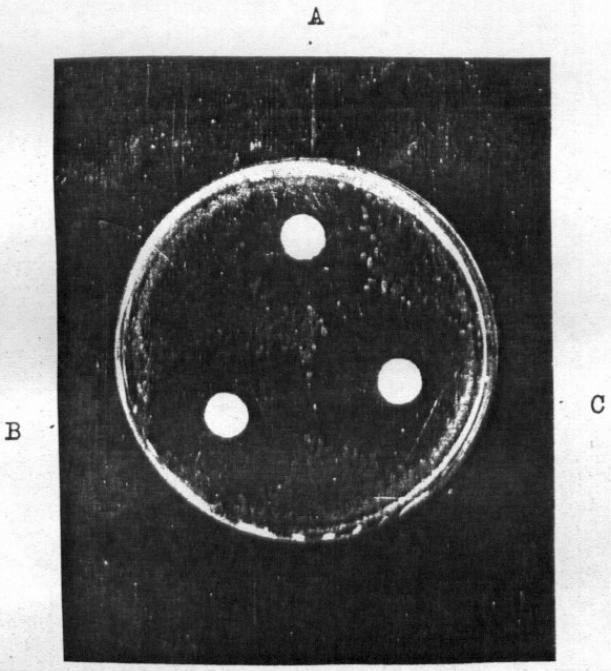



Fig. (4): Effect of Homai-80 on the growth of Streptomyces scabies using disc diffusion method at different concentrations.

A = 50 µg/disc.
B = 100 µg/disc.
C = 150 µg/disc.

Table (3): The least bactericidal concentrations (LCC) of Homai-80 and Streptomycin against phytopathogenic bacteria using poisoned agar method .

| Phytopathogenic bacteria | Minimal inhibitory concentration (ppm) | |
|---|---|---|
| | Homai-80 | Streptomycin |
| Agrobacterium tumefaciens | 25-30 | 100-200 |
| Erwinia atroseptica | 20-25 | < 1.0 |
| Erwinia carotovora | 20-25 | < 1.0 |
| Streptomyces scabies | < 1.0 | < 1.0 |

the bactericidal effect of Homai-80 on all of the tested bacteria.

(2) Streptomycin, compared with Homai-80, had similar low L.C.C. in the case of Streptomyces scabies (i.e. $<1.0$ ppm) and relatively lower L.C.C. in cases of Erwinia atroseptica and E. carotovora (i.e. $<1.0$ ppm). On the other hand, Streptomycin had relatively higher L.C.C. than Homai-80 in the case of Agrobacterium tumefaciens where the minimal inhibitory concentration landed between 100 and 200 ppm.

2- Effect of antagonistic isolates on growth of the tested phytopathogenic bacteria:

(A) Selection of bacterial isolates antagonistic to the tested phytopathogenic bacteria:

The initial screening of more than 500 bacterial colonies originated from different rhizosphere-soil samples resulted in the isolation of 31 different bacterial isolates exhibiting obvious antibiosis against one or more of the tested phytopathogenic bacteria. Fig. (5) shows one of the plates used in the initial screening.

Each of the selected isolates was tested for purity and designated with a code number. Preliminary examination

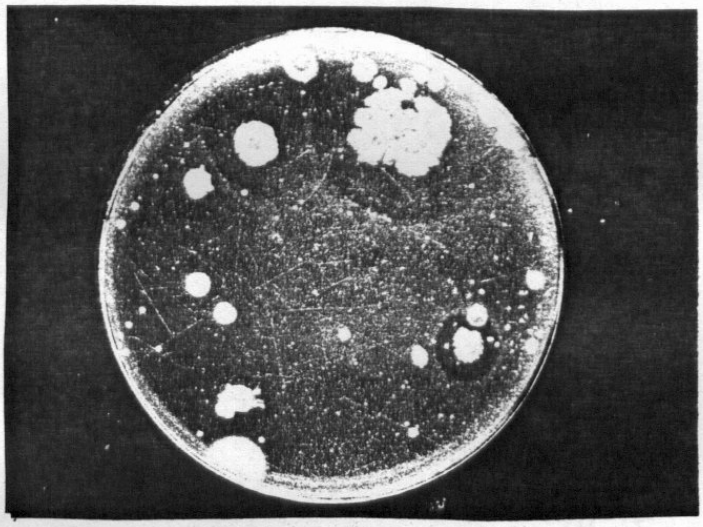


Fig. (5): Initial screening of antagonistic isolates against the tested phyto-pathogenic bacteria.

indicated that 28 of the antagonistic isolates were aerobic spore-formers, whereas 3 isolates were actinomycetes.

(B) Estimation of the efficiency of the antagonistic isolates:

The efficiency of each of the selected antagonistic isolates against the tested phytopathogenic bacteria were determined using a standardized test. Results presented in Table (4) indicate that :

(1): Some of antagonistic isolates had a limited inhibitory spectrum (e.g. isolate $B_{12}$ which inhibited only Agrobacterium tumefaciens, isolates $B_{36}$ and $S_{42}$ which inhibited only Erwinia carotovora , and isolate $S_{44}$ which inhibited Streptomyces scabies only). On the other hand, some of the antagonists had such a wide spectrum of inhibitory action that, they could inhibit all of the tested phytopathogenic bacteria (e.g. isolates $B_{VI}$, $B_{VIII}$, $B_1$, $B_3$, $B_4$, $B_5$, $B_7$, $B_8$, $B_9$, $B_{11}$, $B_{37}$, $B_{38}$, $B_{39}$, and $B_{43}$ ).

(2): Among wide-spectrum isolates, the spore-former isolates $B_1$ and $B_{43}$ proved to have the highest efficiency against

Table (4): Efficiency of antagonistic isolates against phytopathogenic bacteria.

| Phytopathogenic bacteria | | Antagonistic bacteria: Spore - former rods | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Actinomycetes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $B_I$ | $B_{II}$ | $B_{III}$ | $B_{VI}$ | $B_{VIII}$ | $B_1$ | $B_{12}$ | $B_3$ | $B_4$ | $B_5$ | $B_6$ | $B_7$ | $B_8$ | $B_9$ | $B_{101}$ | $B_{102}$ | $B_{11}$ | $B_{30}$ | $B_{31}$ | $B_{32}$ | $B_{34}$ | $B_{36}$ | $B_{37}$ | $B_{38}$ | $B_{39}$ | $B_{43}$ | $B_{45}$ | $B_{C1}$ | $S_{41}$ | $S_{42}$ | $S_{..}$ |
| Agrobacterium tumefaciens | C | | | | 12 | 10 | 7 | 4 | 9 | 8 | 10 | | 9 | 11 | 11 | | | 11 | | | | 3 | | 17 | 5 | 3 | 5 | | | | | |
| | Z | | | | 26 | 32 | 27 | 15 | 24 | 28 | 26 | | 28 | 30 | 27 | | | 26 | | | | 8 | | 35 | 8 | 7 | 26 | | | | | |
| | Z/C | | | | 2.2 | 3.2 | 3.9 | 3.8 | 2.7 | 3.5 | 2.6 | | 3.1 | 2.7 | 2.5 | | | 2.4 | | | | 2.7 | | 2.1 | 1.6 | 2.3 | 5.2 | | | | | |
| Erwinia atroseptica | C | 6 | 9 | 10 | 8 | 10 | 9 | | 11 | 11 | 10 | 15 | 11 | 10 | 9 | 10 | 11 | 9 | 11 | | | | | 4 | 7 | 6 | 6 | 6 | 10 | 7 | | |
| | Z | 17 | 17 | 17 | 24 | 28 | 27 | | 28 | 29 | 28 | 33 | 29 | 27 | 27 | 27 | 28 | 27 | 22 | | | | | 12 | 9 | 10 | 14 | 16 | 16 | 25 | | |
| | Z/C | 2.8 | 1.9 | 1.7 | 3.0 | 2.8 | 3.0 | | 2.6 | 2.6 | 2.8 | 2.2 | 2.6 | 2.7 | 3.0 | 2.7 | 2.6 | 3.0 | 2.0 | | | | | 3.0 | 1.3 | 1.7 | 2.3 | 2.7 | 1.6 | 3.6 | | |
| Erwinia carotovora | C | 10 | 13 | 15 | 13 | 12 | 10 | | 9 | 10 | 13 | 13 | 11 | 12 | 12 | 9 | 9 | 12 | 8 | 13 | 13 | 5 | 6 | 25 | 6 | 5 | 6 | 26 | 13 | 5 | 7 | |
| | Z | 20 | 22 | 25 | 32 | 30 | 29 | | 26 | 29 | 32 | 27 | 29 | 30 | 30 | 22 | 25 | 31 | 23 | 26 | 28 | 12 | 18 | 37 | 14 | 12 | 15 | 38 | 22 | 21 | 16 | |
| | Z/C | 2.0 | 1.7 | 1.7 | 2.5 | 2.5 | 2.9 | | 2.9 | 2.9 | 2.5 | 2.1 | 2.6 | 2.5 | 2.5 | 2.4 | 2.8 | 2.6 | 2.9 | 2.0 | 2.2 | 2.4 | 3.0 | 1.5 | 2.3 | 2.4 | 2.5 | 1.9 | 1.7 | 4.2 | 2.3 | |
| Streptomyces scabies | C | 10 | 12 | 11 | 14 | 9 | 11 | | 13 | 13 | 14 | 16 | 13 | 13 | 14 | 12 | 14 | 14 | 12 | 11 | 7 | 5 | | 21 | 5 | 6 | 22 | 19 | 8 | 4 | | |
| | Z | 27 | 32 | 25 | 28 | 27 | 33 | | 37 | 36 | 37 | 37 | 35 | 36 | 36 | 30 | 31 | 37 | 33 | 31 | 22 | 16 | | 43 | 14 | 18 | 44 | 44 | 20 | 32 | | |
| | Z/C | 2.7 | 2.7 | 2.3 | 2.0 | 3.0 | 3.0 | | 2.8 | 2.8 | 2.6 | 2.3 | 2.7 | 2.8 | 2.6 | 2.5 | 2.2 | 2.6 | 2.8 | 2.8 | 3.1 | 3.2 | | 2.0 | 2.8 | 3.0 | 2.0 | 2.3 | 2.5 | 8.0 | | 4 |
| a.r.p.a. | | 1.9 | 1.6 | 1.4 | 2.4 | 2.9 | 3.2 | 0.9 | 2.8 | 2.9 | 2.6 | 1.7 | 2.8 | 2.7 | 2.7 | 1.9 | 1.9 | 2.7 | 1.9 | 1.2 | 1.3 | 1.6 | 0.8 | 2.2 | 2.0 | 2.4 | 3.0 | 1.7 | 1.5 | 3.9 | 0.6 | 1. |

C = Diameter of antagonistic colony (in mm.) after 48 hr. incubation.

Z = Diameter of inhibition zone (in mm.) after 48 hr. from spraying.

Z/C = Relative power of antibiosis.

a.r.p.a.= Average relative power of antibiosis.

the tested phytopathogenic bacteria since the spectrum of affected phytopathogenic bacteria comprised all of the tested pathogens. Isolates $B_1$ and $B_{43}$ showed the highest average value of relative power of antibiosis (3.2 and 3.0, respectively).

(3) Among actinomycetes, isolate $S_{41}$, showed the highest average value of relative power of antibiosis although it had no antagonistic effect against Agrobacterium tumefaciens. So, isolates $B_1$, $B_{43}$ and $S_{41}$ were considered to have the highest antagonistic efficiency against the tested phytopathogenic bacteria, and were subjected to identification tests and pot experiments.

Figs. 6, 7, 8 and 9 show sensitivity of each phytopathogenic bacterium towards some antagonistic isolates.

(C) Identification of antagonistic isolates:

Preliminary examination of the thirty-one antagonistic isolates indicated that 28 of them were aerobic sporeformers, whereas 3 isolates were actinomycetes. For the most efficient isolates ( i. e. $B_1$ , $B_{43}$ , and $S_{41}$ ) , cultural, morphological, and physiological properties were tested in order to nominate these organisms according to

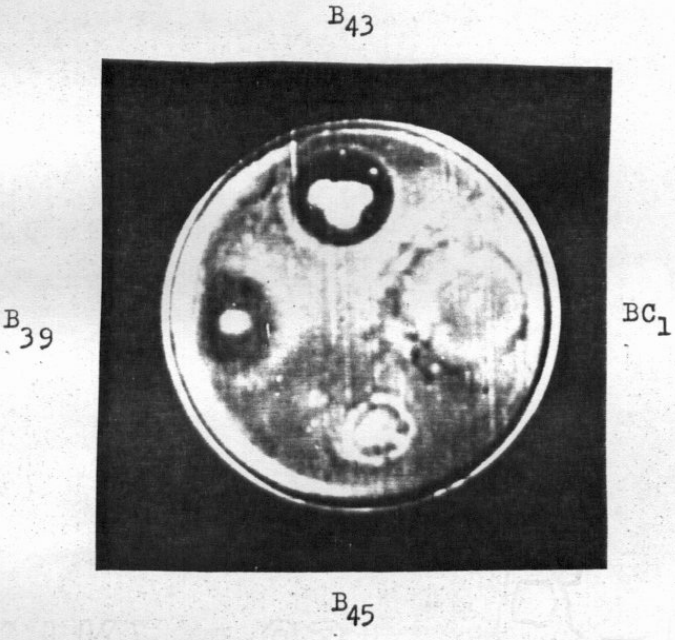



Fig. (6): Sensitivity of Agrobacterium tumefaciens towards some antagonistic isolates (antagonistic isolates are designated with code numbers).

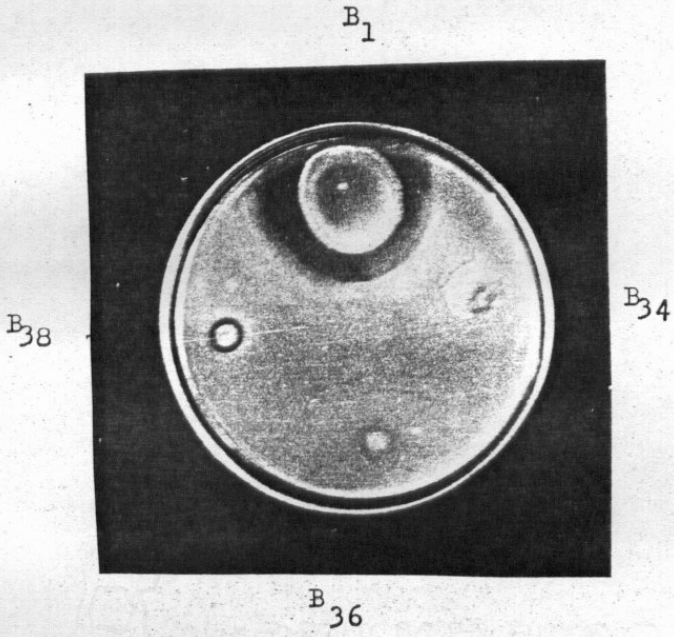



Fig. (7): Sensitivity of Erwinia atroseptica towards some antagonistic isolates (antagonistic isolates are designated with code numbers).

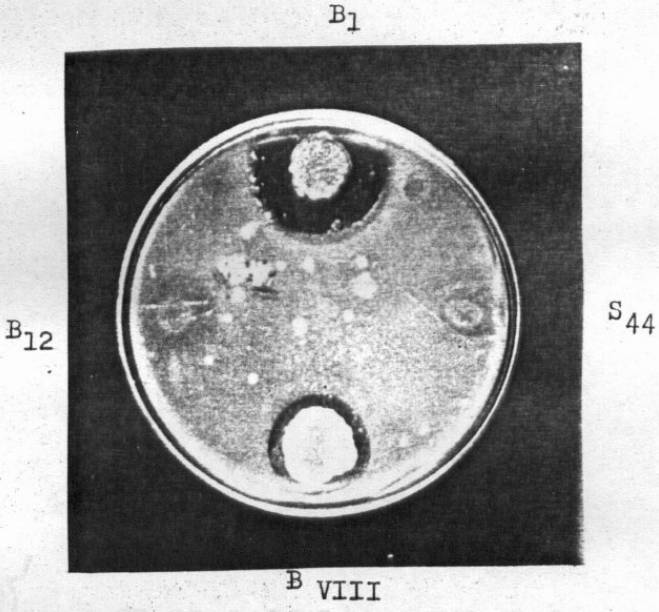



Fig. (8): Sensitivity of Erwinia carotovora towards some antagonistic isolates (antagonistic isolates are designated with code numbers).

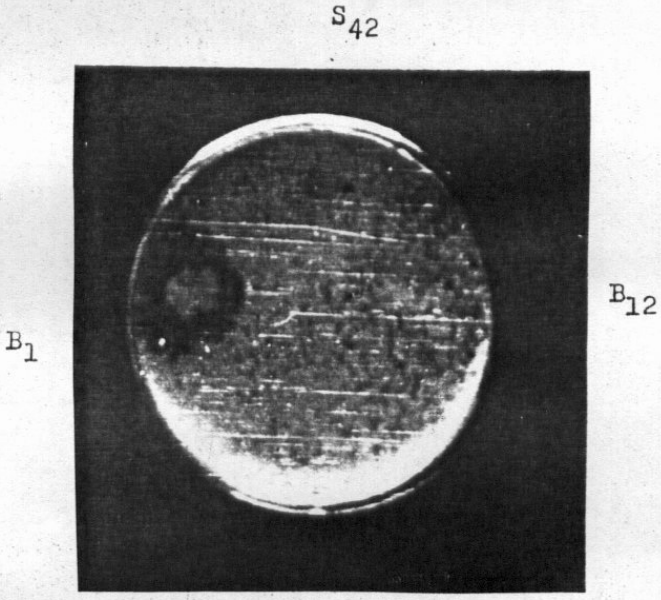



Fig. (9): Sensitivity of Streptomyces scabies towards some antagonistic isolates (antagonistic isolates are designated with code numbers).

Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974). Tables (5), (6), and (7) present the data obtained in these experiments.

Comparing the data given in Tables 5, 6, and 7 with those reported by different investigators (Buchanan & Gibbons, 1974 and Smith et al., 1952), the following could be concluded :

i) Isolate $B_1$ showed morphological and biochemical characteristics (Table 5) identical to those known for Bacillus megaterium de Bery.

ii) Isolate $B_{43}$ showed morphological and biochemical characteristics (Table 6) identical to those known for Bacillus brevis Migula.

iii) Isolate $S_{41}$ showed morphological and biochemical characteristics (Table 7) similar to Streptomyces sp. Waksman and Henrici .

Table (5): Morphological characteristics and biochemical activities of the antagonistic isolate $B_1$

| Test | Result |
|---|---|
| Shape of cells | Rods, single or in short chain |
| Size of cells on glucose agar | 1.2 x 2.1 µm |
| Sporulation | Cylindrical, central. |
| Motility | Motile |
| Gram reaction | + |
| Catalase activity | + |
| Aerobiosis | + |
| Indole formation | - |
| Gelatin liquefaction | + |
| Casein hydrolysis | + |
| Nitrate reduction | -/± |
| Starch hydrolysis | + |
| Acetyl methyl carbinol production (V.P.) | - |
| Growth in: 7% NaCl | + |
| 10% NaCl | + |
| Growth on : 56 °C | - |
| 65 °C | - |
| Products of action on glucose: | |
| Acid | -/± |
| Gas | - |
| Anaerobic growth on glucose | - |
| Citrate utilization | + |
| Urea decomposed | - |
| Egg yolk reaction | + |
| Gas production from nitrate under anaerobic condition | - |

+ = positive.
- = negative.
-/± = slight positive.

Table (6): Morphological characteristics and biochemical activities of the antagonistic isolate $B_{43}$

| Test | Result |
|---|---|
| Shape of cells | Rods, single or in short chain. |
| Size of cells on glucose agar | 0.6 - 0.8 x 1.6 µm. |
| Sporulation | Elliptical with distended sporangium |
| Motility | Motile |
| Gram reaction | + |
| Catalase activity | + |
| Aerobiosis | + |
| Indole formation | - |
| Gelatin liquefaction | + |
| Casein hydrolysis | + |
| Nitrate reduction | + |
| Starch hydrolysis | - |
| Acetyl methyl carbinol production (V.P.) | - |
| Growth in : 7% NaCl | - |
| 10% NaCl | - |
| Growth on : 56 °C | - |
| 65 °C | - |
| Products of action on glucose: | |
| Acid | -/± |
| Gas | - |
| Anaerobic growth on glucose | -/± |
| Citrate utilization | + |
| Urea decomposed | - |
| Egg yolk reaction | + |
| Gas production from nitrate under anaerobic condition | - |

+ = positive.
- = negative.
-/± = slight positive.

Table (7): Morphological characteristics and biochemical activities of the antagonistic isolate $S_{41}$.

| Test | Result |
|---|---|
| Colonies | Initially small, discrete, lichenoid; and smooth surfaced colonies, which turn powdery at later stages. |
| Mycelium | Well developed branched mycelium carrying cylinderical spores in long straight chains. |
| Spore color *en masse* | Gray |
| Production of melanoid pigment | + |
| Aerobiosis | + |
| Gram reaction | + |
| Nitrate reduction | + |
| Gelatin liquefaction | + |
| Casein hydrolysis | + |
| Starch hydrolysis | + |

+ = Positive .

3- Antibiosis between phytopathogenic bacteria:

This experiment aimed to determine whether the tested phytopathogenic bacteria could inhibit each other. Data presented in Table (8) show that there was no inhibitory action of any of the tested phytopathogenic bacteria against each other,and all of them were capable to survive side by side.

Table (8): Antibiosis between the tested phyto-pathogenic bacteria.

| Phytopathogenic bacteria | A. tumefaciens | E. atroseptica | E. carotovora | S. scabies |
|---|---|---|---|---|
| Agrobacterium tumefaciens | | - | - | - |
| Erwinia atroseptica | - | | - | - |
| E. carotovora | - | - | | - |
| Streptomyces scabies | - | - | - | |

- = no antibiosis .

II- In vivo experiments:

The fungicide "Homai-80" and the antagonistic isolates $B_1$, $B_{43}$ and $S_{41}$ proved in vitro experiments to be highly effective against Agrobacterium tumefaciens, Erwinia atrosptica, E. carotovora and Streptomyces scabies. Therefore, pot experiments were conducted to evaluate their efficacy to control the diseases caused by those phyto-pathogenic bacteria.

1- Crown-gall disease:

Results presented in Table (9) show that chemical treatment of sunflower transplants with"Homai-80" at the rate of 0.25% and 0.50% prior to the infection with Agro-bacterium tumefaciens significantly inhibited the gall formation. Percentages of gall inhibition (G.I.P.) measured 4 weeks after transplanting were determined to be about 76% and 83 %, respectively. There was no significant difference between the two used preparations (0.25% and 0.50%) of "Homai-80" on the formation of galls.

Significant but lower inhibition of disease incidence could also be achieved upon treatment of the transplants with suspensions containing $10^6$ - $10^8$ cfu/ml of the antagonistic isolates $B_1$ (Bacillus megaterium) or $B_{43}$ (B. brevis) prior to the infection with A. tumefaciens. Percentages of gall

Table (9): Effect of chemical treatment with "Homai-80" and treatment with the antagonistic isolates $B_1$ and $B_{43}$ prior to the infection with A. tumefaciens on the incidence of crown-gall disease on sunflower seedlings .

| Treatment | Concentration or cell density | Mean of gall wt.* gm/plant | G.I.P.** |
|---|---|---|---|
| A. tumefaciens | $10^7$ cfu/ml | 1.23 a | |
| Isolate $B_1$ | $10^8$ cfu/ml | 0.0 | |
| Isolate $B_{43}$ | $10^8$ cfu/ml | 0.0 | |
| Homai-80 | 0.25 % | 0.30 bd | 75.9 |
| + | 0.50 % | 0.21 b | 82.9 |
| A. tumefaciens | $10^7$ cfu/ml | | |
| Isolate $B_1$ : | $10^6$ : $10^7$ cfu/ml | 0.66 c | 46.5 |
| A. tumefaciens | $10^7$ : $10^7$ " | 0.71 c | 42.1 |
| | $10^8$ : $10^7$ " | 0.60 c | 51.0 |
| Isolate $B_{43}$ : | $10^6$ : $10^7$ cfu/ml | 0.43 d | 64.8 |
| A. tumefaciens | $10^7$ : $10^7$ " | 0.43 d | 64.9 |
| | $10^8$ : $10^7$ " | 0.44 d | 64.5 |

* Gall weights were determined 4 weeks after transplanting.

** G.I.P. = gall inhibition percentage.

*** Means designated by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

inhibition were about 47% and 65%, respectively. In both of the antagonistic isolates, there were no significant differences between the three used cell densities ($10^6$, $10^7$ and $10^8$ cfu/ml) on gall formation. Samples of the different treatments are illustrated in Fig. (10).

2- Potato black-leg disease:

Results presented in Table (10) show that treatment of potato seed-pieces with "Homai-80" before infection with Erwinia atroseptica increased the developing healthy plants. Seed-pieces treated with 0.25% and 0.50% preparations of "Homai-80" produced 80% and 100% healthy plants, respectively; whereas in the check treatment(seed-pieces treated with E. atroseptica only), no plant free from black-leg symptoms could develop during 6 weeks after planting.

Treatment of seed-pieces with suspensions containing $10^6$ - $10^8$ cfu /ml of the antagonistic isolate $S_{41}$ (Streptomyces sp.) prior to the infection with E. atroseptica gave the highest efficacy (100%) . All of the treated seed-pieces developed to healthy plants.

Lower efficacy was obtained upon treatment with the antagonistic isolate $B_1$ (Bacillus megaterium) . It ranged between 60-80% according to the cell density of the antagonistic isolate. Higher cell densities resulted in

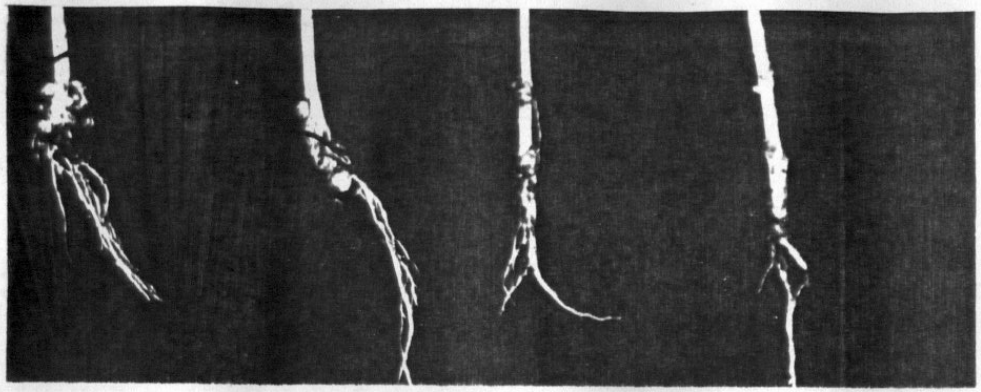



Fig. (10): Gall formation on sunflower seedlings treated with :
A) Agrobacterium tumefaciens (control).
B) Antagonistic isolate $B_1$ + A. tumefaciens.
C) Antagonistic isolate $B_{43}$ + A. tumefaciens.
D) "Homai-80" + A. tumefaciens .

Table (10): Incidence of black-leg disease as affected by treatment of potato seed-pieces with "Homai-80" and with the antagonistic isolates $B_1$ and $S_{41}$ prior to the infection with E.atroseptica.

| Treatment | Concentration or cell density | From 10 pots * Not emerged | Diseased plants | Healthy plants | Efficacy of ** treatment % |
|---|---|---|---|---|---|
| Not treated | | 0.0 | 0.0 | 10 | |
| E.atroseptica | $10^7$ cfu/ml | 2 | 8 | 0.0 | |
| Homai-80 | 0.25 % | 0.0 | 0.0 | 10 | |
| | 0.50 % | 0.0 | 0.0 | 10 | |
| Isolate $B_1$ | $10^8$ cfu/ml | 2 | 0.0 | 8 | |
| Isolate $S_{41}$ | $10^8$ cfu/ml | 0.0 | 0.0 | 10 | |
| Homai-80 | 0.25 % | 0.0 | 2 | 8 | 80 |
| + | 0.50 % | 0.0 | 0.0 | 10 | 100 |
| E.atroseptica | $10^7$ cfu/ml | | | | |
| Isolate $B_1$ : | $10^6$:$10^7$ cfu/ml | 1 | 1 | 8 | 80 |
| E.atroseptica | $10^7$:$10^7$ " | 1 | 1 | 8 | 80 |
| | $10^8$:$10^7$ " | 3 | 1 | 6 | 60 |
| Isolate $S_{41}$ : | $10^6$:$10^7$ cfu/ml | 0.0 | 0.0 | 10 | 100 |
| E.atroseptica | $10^7$:$10^7$ " | 0.0 | 0.0 | 10 | 100 |
| | $10^8$:$10^7$ " | 0.0 | 0.0 | 10 | 100 |

* Data were taken weekly for 6 weeks after planting.

** Efficacy of treatment =

$$\frac{\%\text{ healthy plants in treatment} - \%\text{ healthy plants in control}}{\%\text{ healthy plants in treatment}} \times 100$$

lower efficacy, presumably via their effect on emergence of seed-pieces. This adverse effect on emergence was detectable in all seed-pieces treated with isolate $B_1$ (B. megaterium) only.

3- Soft-rot disease:

Results presented in Table (11) indicate that treatment of potato seed-pieces with "Homai-80" at the rate of 0.25% and 0.50% prior to the infection with Erwinia carotovora protected them against rotting . Percentage of emergence increased and reached 100%.

Comparable efficacy could be achieved by treatment of seed-pieces with the antagonistic isolate $S_{41}$ (Streptomyces sp.). The adverse effect of isolate $B_1$ (Bacillus megaterium) on the emergence of the seed pieces was also observed in this experiment , and lower efficacy of the treatment (60-80%) were obtained.

4- Common scab disease of potato:

Results presented in Table (12) show that soil treatment with "Homai-80" at the rate of 2.5 and 5 g/kg prior to the soil infestation with Streptomyces scabies completely suppressed the incidence of scab. However, deleterious effects were observed on the developing plants grown in soil

Table (11): Effect of chemical treatment with "Homai-80" and treatment with the antagonistic isolates $B_1$ and $S_{41}$ prior to the infection with E. carotovora on the incidence of soft-rot disease in potato seed-pieces.

| Treatment | Concentration or cell density | From 10 pots* | | Efficacy of ** treatment |
|---|---|---|---|---|
| | | Not emerged (rotted) | Emerged (healthy) | % |
| Not treated | | 0.0 | 10 | |
| E.carotovora | $10^7$ cfu/ml | 10 | 0.0 | |
| Homai-80 | 0.25 % | 0.0 | 10 | |
| | 0.50 % | 0.0 | 10 | |
| Isolate $B_1$ | $10^8$ cfu/ml | 2 | 8 | |
| Isolate $S_{41}$ | $10^8$ cfu/ml | 0.0 | 10 | |
| Homai-80 | 0.25 % | 0.0 | 10 | 100 |
| + | 0.50 % | 0.0 | 10 | 100 |
| E.carotovora | $10^7$ cfu/ml | | | |
| Isolate $B_1$ : | $10^6$:$10^7$ cfu/ml | 2 | 8 | 80 |
| E.carotovora | $10^7$:$10^7$ " | 4 | 6 | 60 |
| | $10^8$:$10^7$ " | 4 | 6 | 60 |
| Isolate $S_{41}$: | $10^6$:$10^7$ cfu/ml | 0.0 | 10 | 100 |
| E.carotovora | $10^7$:$10^7$ " | 0.0 | 10 | 100 |
| | $10^8$:$10^7$ " | 0.0 | 10 | 100 |

* Data were taken weekly for 6 weeks after planting.

** Efficacy of treatment =

$$\frac{\%\text{ emergent seed-pieces in treatment} - \%\text{ emergent seed-pieces in control}}{\%\text{ emergent seed-pieces in treatment}} \times 100$$

Table (12): Effect of soil treatment with "Homai-80" and with the antagonistic isolates $B_1$ and $S_{41}$ on the common-scab incidence and yield of potato grown in soil infected with *S. scabies*.

| Treatment | Concentration or cell density | * D.I.P. | Yield (gm/pot) |
|---|---|---|---|
| Not treated | | 0.0 | 160 b |
| *S. scabies* | $10^7$ cfu/ml | 20.3 a | 175 b |
| Homai-80 | 2.5 gm/kg | | 140 b |
| | 5.0 " | | 80 a |
| Isolate $B_1$ | $10^8$ cfu/ml | 0.0 | 155 b |
| Isolate $S_{41}$ | $10^8$ cfu/ml | 0.0 | 166 b |
| Homai-80 | 2.5 gm/kg | 0.0 e | 151 b |
| + | 5.0 " | 0.0 e | 85 a |
| *S. scabies* | $10^7$ cfu/ml | | |
| Isolate $B_1$ : | $10^6$:$10^7$ cfu/ml | 6.1 c | 165 b |
| *S. scabies* | $10^7$:$10^7$ " | 5.8 c | 161 b |
| | $10^8$:$10^7$ " | 6.2 c | 168 b |
| Isolate $S_{41}$ : | $10^6$:$10^7$ cfu/ml | 9.4 b | 171 b |
| *S. scabies* | $10^7$:$10^7$ " | 9.1 b | 169 b |
| | $10^8$:$10^7$ " | 10.3 b | 171 b |

* D.I.P. = disease index percentage.

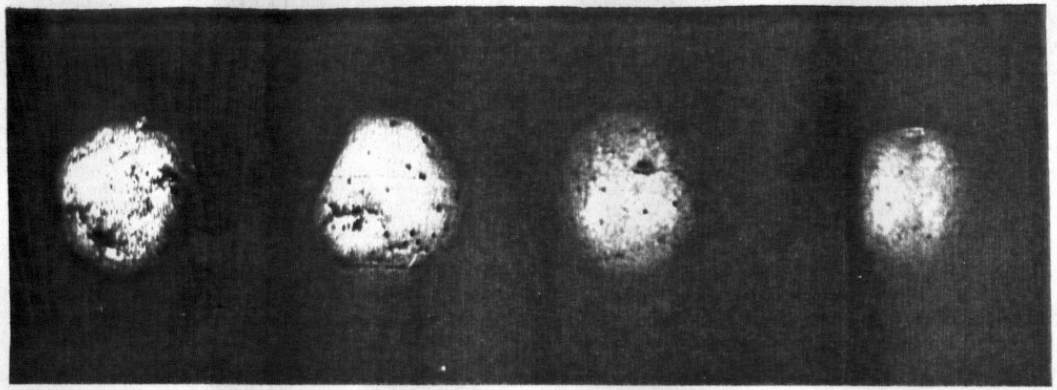
** Soil treatment with "Homai-80" was carried out prior to planting.

*** Soil inoculation with the antagonistic isolates and/or infestation with *S. scabies* were carried out immediately after planting .

**** Means designated by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range test.

treated with the higher dose of "Homai -80" (5 g/kg). Plants were deformed, and the yield of tubers was significantly lower.

Inoculation of soil with the antagonistic isolate $B_1$ (Bacillus megaterium) significantly reduced the disease index percentage (D.I.P.). This D.I.P. became about 6 % whereas in the check treatment (soil infested with S. scabies only), it reached about 20 % . The same result, but to a lesser extent, was observed upon inoculation with the antagonistic isolate $S_{41}$ (Streptomyces sp.) where disease index percentages were about (9 - 10 %). Inoculation of soil with either isolate $B_1$ or isolate $S_{41}$ did not adversely affect the yield of tubers. Figure (11) illustrates samples of different treatments.



A B C D

Fig. (11): Common-scab incidence on tubers of potato grown in soil treated with :

A) Streptomyces scabies (control).
B) Antagonistic isolate $S_{41}$ + S. scabies .
C) Antagonistic isolate $B_1$ + S. scabies .
D) "Homai-80" + S. scabies .

## DISCUSSION

It has been suggested by many investigators that certain groups of fungicides might be valuable in the control of certain bacterial diseases (El-Kazzaz,1966; Shneider et al., 1968 ; Rushdi et al., 1972; Shihata, 1974 and Seif El-Yazal, 1980) . Moreover, promising informations have been obtained about the use of fungicides in the control of combined infections with bacteria and fungi (Crossan, 1959; Line and Eide, 1960; Robinson et al., 1960; and Weingartner and Shumaper, 1974).

Other investigators suggested the adventitious inhibition action of herbicides on microbial phytopathogenic agents (Altman and Campbell, 1977). So, on the aforementioned concepts, the first aim of this study "Chemical control" was built. Therefore, the effect of 5 fungicides (Benlate, Homai-80, Rovral, Tecto, and Vitavax/Captan) and 5 pre-emergence herbicides (Bladex Enide, Eptam, Liuron and Sencor) were tested In vitro against Agrobacterium tumefaciens, Erwinia atroseptica, Erwinia carotovora and Streptomyces scabies the causal agents of crown gall, potato black leg, soft rot and potato common scab diseases,respectively.

Although the tested herbicides showed no or negligible direct inhibitory effect upon the growth of the

tested phytopathogenic bacteria, this result, however, resembles an attempt, which may be of importance in the field of utilizing herbicides as antiphytopathogenic bacterial agents. The remisseness of the used herbicides to have direct effect on the test phytopathogenic bacteria is not surprising in light of reports declaring that population of microorganisms including Pseudomonas-like bacteria increase in soil receiving herbicides (Carter & Camper, 1973 ; Ulasevich, 1978). Furthermore, numerous reports suggested the microbiological degradation and utilization of herbicides as carbon and nitrogen sources by bacterial isolates (Mickovski, 1970; Srirama Raju and Rangaswami, 1971; Carter & Camper , 1973; Rosenburg & Alexander, 1978, Walker, 1978; and Berry et al., 1979).

On the other hand, results of In vitro experiments indicated that the fungicide "Homai-80" surpassed all the tested chemicals concerning the inhibitory effect on the growth of the phytopathogenic bacteria under investigation. Although "Homai-80" was inferior to the antibiotic streptomycin in this respect, its application in pot experiments as pre-planting seed-pieces, seedlings, or soil treatments showed obvious effectiveness in controlling crown gall, potato black leg, soft rot and potato common scab diseases. Dipping the lower part of sunflower

seedlings for 5 min. in 0.25 % and 0.50% aqueous solutions of "Homai-80"prior to the infection with A. tumefaciens inhibited about 76% and 83% of galls formed on the infected seedlings , respectively. More effective protection (100%) could be achieved against potato black leg disease by dipping the seed-pieces for 5 min. in 0.50% aqueous solution of "Homai-80" prior to the infection with E. atroseptica. "Homai-80" exhibited similar efficiency (100%) against soft rot disease caused by E. carotovora even at lower concentration (0.25%). No symptoms of common scab disease could be detected on potato tubers grown in soil simultaneously infested with S. scabies and treated with "Homai-80".

The economic beneficial use of "Homai-80" in controlling the bacterial diseases under investigation seems to be hopeful, since beside its effectiveness, it is also well tolerated by plant. No symptoms of toxicity were detected on sunflower and potato plants developed from seedlings and seed-pieces dipped for 5 min. in 0.25% or 0.50% aqueous solutions of it. However, malformation of leaves and decreased tuberance were observed on potato grown in soil treated with high doses (5 g/kg) of "Homai-80". These deleterious effect were not detectable at lower doses (e.g. 2.5 g/kg). Since there were no significant differences between the two used doses (2.5 g/kg

and 5 g/kg) on the incidence of common scab disease, this might lead us to suggest 2.5 g/kg as a suitable dose for soil treatment. However, further informations about the persistence of "Homai-80", its effect on soil microflora, and toxicity to mammals are necessary before it can be recommended for general use.

Beside efficiency, control of plant diseases must be more concerned with improving and maintaining human health and environment. We were and still depending on chemicals for control of plant diseases despite the threatening reports about the extensive use of chemicals in this field. Residues of these chemicals on plants may carry over into foodstuffs and many become health hazardous or may accumulate in soil and disturb its biological equilibrium. One of the potential solutions of this problem would be the exploitation of nature's biological potency to have sufficient biocontrol of phytopathogens with no toxic residues, safe for application and also cheap in cost.

These goals in mind, in addition to the frequent reports implicating antagonists as a factor influencing the pests and plant pathogens (Backer & Cook, 1974 ; and Burges , 1981), prompted proceeding the second part of our study as "biological control". Therefore,several

hundreds rhizosphere-soil samples collected from different locations were screened for antagonists against the phytopathogenic bacteria under investigation . Thirty-one bacterial isolates exhibiting marked antibiosis against one or more of the tested phytopathogenic bacteria were isolated. The predominant antagonists isolated were Bacillus and Actinomycetes bacterial types. This result is in agreement with numerous reports revealing the efficiency of the two types as microbial antagonists (El-Leithy & Monib, 1961; Weinhold & Bowman, 1968; Cooksey & Moore, 1979 ; Mahmoud et al., 1981 ;and Neweigy et al., 1982).

The antagonistic isolates obtained in the preliminary screening were subjected to standardized tests to select those having the highest efficiency against the phytopathogenic bacteria under investigation. Criteria considered in the efficiency estimation were :

a) spectrum of the affected phytopathogenic bacteria, and b) relative power of antibiosis against each pathogen. The isolates $B_1$, $B_{43}$ and $S_{41}$, which were identified as Bacillus megaterium, B. brevis and Streptomyces sp., respectively, proved In vitro to have the highest efficiency suggesting the beneficial employment of these isolates in controlling the bacterial diseases under investigation .

Results of pot experiments confirmed the aforementioned suggestion. Treatment of the lower part of sunflower transplants with cell suspension of isolate $B_{43}$ (B. brevis) prior to the infection with A. tumefaciens reduced galling by about 65% . The level of control achieved with isolate $B_{43}$ (B. brevis) is somewhat lower compared to that reported by New & Kerr (1972) and Cooksey & Moore (1980) using strain 84 of Agrobacterium radiobacter . However, isolate $B_{43}$ (B. brevis) may be of importance for control of A. tumefaciens strains not subject to control by strain 84. Some strains of A. tumefaciens were reported to be insensitive to the bacteriocin (Agrocin 84) produced In vitro by strain 84 (Kerr & Panagopoulos , 1977) . It is plausible that such strains may be sensitive to other bacteriocins having different chemical structures . Agrocin 84 was identified as a phosphoramidate of an adenine deoxyarabinoside (Roberts et al., 1977) . B. brevis , to which isolate $B_{43}$ belongs, was reported to produce a cyclic decapeptide antibiotic (Gramicidin S) which is chemically different from Agrocin 84 (Vandamme & Demain, 1976). However , this speculation remains to be tested.

A complete protection (100%) against black-leg disease of potato could be achieved by dipping seed-pieces

for 5 min. in washed cell suspension of isolate $S_{41}$ (Streptomyces sp.) containing $10^6$ - $10^8$ cfu/ml prior to the infection with E. atroseptica. This isolate ($S_{41}$) seems to have an outstanding efficiency. In case of soft rot disease, treatment of seed-pieces with this isolate as described above prior to the infection with E. carotovora protected the seed-pieces completely against rotting and 100% emergence could be achieved.

Severity of potato common scab could be reduced to about 30% of check treatment, when soil was inoculated with washed cell suspension of isolate $B_1$ (B. megaterium) and simultaneously infested with S. scabies in the ratio of $10^6$-$10^8$ : $10^7$ cfu/ml, respectively. This result is of particular importance, since most available reports implicating Bacilli as an antagonistic factor influencing scab incidence were more or less speculative, (Weinhold & Bowman , 1968; Mahmoud et al., 1981a and 1981b). Some Bacilli , which proved In vitro to be antagonistic to S. scabies failed to control potato common scab (Mohamed, 1974).

Beside the marked efficiency of the three antagonistic isolates $B_1$ (Bacillus megaterium, $B_{43}$ (B. brevis) and $S_{41}$ (Streptomyces sp.) in controlling bacterial disease mentioned before , they are not pathogenic to plants used throughout the experiments;treated

plants were as healthy as untreated plants. An exception was in case of potato seed-pieces treated with isolate $B_1$ (B. megaterium), where about 20% of replicates rotted and failed to emerge. Since this effect was not observed using whole tubers instead of seed-pieces, it can be attributed to the growth of B. megaterium on the wounded surfaces of seed-pieces as well as to the strong starch-hydrolyzing activity characterizing this bacterium.

The results of biological control indicate that control levels achieved in cases of potato black leg and soft rot diseases using the antagonistic isolate $S_{41}$ (Streptomyces sp.) were comparable to those achieved by using "Homai-80". In cases of crown gall and potato common scab diseases, biological control was generally inferior , though satisfactory, to chemical control. This is not surprising since the action of antagonists in contrast to chemicals, is time-dependent. Antagonists have to grow and to be established before they can express their efficiency. In addition, they are subject, as living organisms, to the environmental conditions. Since infection with the pathogens were carried out in our experiments almost directly after treatment with the antagonists, therefore, it is debatable,that control levels achievable could be improved under conditions permitting

better establishment of antagonists before infection with the pathogens.

An additional advantage encouraging the employment of these antagonistic isolates is their long-term persistence in soil as saprophytes or as protectable dormant spores . Seed, seedling, or soil inoculation with such isolates would lead to a predominance of these antagonistic populations in the rhizosphere of developing plants and suppress the phytopathogenic bacterial agents.The success of bacterial colonization programmes,however, will ultimately depend on several aspects such as cost-benefit ratios, wide spread applicability,and development of practical delivery systems.

The present study is a novel attempt offering sufficient evidence about the tremendous possibilities of biological control of bacterial plant diseases. Further studies about the nature of the produced inhibition substance(s) as well as about influence of environmental factors on biological control are essential. Biotechnological studies dealing with mass production, handling and storage of antagonists are also needed in order to realize the concept of biological control.

SUMMARY

The present investigation was planned to study the chemical and biological control of the following phytopathogenic bacteria:- Agrobacterium tumefaciens , the causal agent of crown-gall disease, Erwinia atroseptica, the causal agent of potato black-leg disease, E. carotovora the causal agent of soft-rot disease ; Streptomyces scabies, the causal agent of potato common-scab disease.

For chemical control, experiments were carried out In vitro to study the inhibitory effect of five fungicides and five (pre-emergence) herbicides on the growth of the tested phytopathogenic bacteria. Fungicides were Benlate, Homai-80, Rovral, Tecto, and Vitavax/Captan . Herbicides were Bladex, Enide , Eptam, Linuron , and Sencor. Streptomycin was used for comparison.

Results revealed that the fungicide "Homai-80" was more effective than the other tested chemicals. The minimal inhibitory concentrations of "Homai-80" landed under 1 ppm in the case of S. scabies and was between 20-25 ppm in the cases of E. atroseptica and E. carotovora. "Homai-80" exhibited a stronger inhibitory effect even than Streptomycin in the case of A. tumefaciens , where the minimal inhibitory concentrations were 20-25 ppm and 100-200 ppm, respectively.

For biological control, screening of several hundreds rhizosphere-soil samples collected from different locations resulted in the isolation of 31 bacterial isolates exhibiting antagonisms In vitro against the tested phytopathogenic bacteria . Twenty-eight of these antagonistic isolates belonged to the rod-shaped, spore-former bacteria and the remain four isolates belonged to Actinomycetes. Isolates $B_1$, $B_{43}$, and $S_{41}$ which showed the highest antagonistic efficiency were identified as Bacillus megaterium, B. brevis, and Streptomyces sp. , respectively according to their morphological,cultural, and biochemical characteristics.

Pot experiments were carried out to determine the efficacy of "Homai-80" and of the isolates $B_1$, $B_{43}$ and $S_{41}$ in decreasing the incidence of the bacterial diseases caused by the tested phytopathogenic bacteria.

In case of crown gall disease, dipping the lower part of sunflower transplants for 5 min.in 0.25% and 0.50 % aqueous solutions of "Homai-80" prior to infection with A. tumefaciens resulted in gall inhibition of about 76% and 83%, respectively. Lower levels of control were achieved using the antagonistic isolates $B_1$ and $B_{43}$; dipping of the lower part of transplants for 5 min. in suspension of $B_1$ and $B_{43}$ containing $10^6$-$10^8$ cfu/ml prior to the infection reduced galling by about 46% and 65%,respectively.

In case of potato black leg disease, dipping of seed-pieces for 5 min. in 0.25% and 0.50% aqueous solutions of "Homai-80" prior to infection with E. atroseptica achieved the healthy development of 80% and 100 % of infected seed-pieces, respectively. Comparable levels of control could be obtained using the antagonistic isolates $B_1$ and $S_{41}$; dipping of seed-pieces for 5 min. in suspensions of $B_1$ and $S_{41}$ containing $10^6$-$10^8$ cfu / ml prior to the infection caused the healthy development of about 73% and 100% of infected seed-pieces, respectively.

In case of soft rot disease, potato seed-pieces could be completely protected against rotting by dipping them for 5 min. in 0.25% aqueous solution of "Homai-80" prior to infection with E. carotovora. Comparable level of control could also be obtained using the antagonistic isolate $S_{41}$; 100% emergence could be achieved by dipping of seed-pieces for 5 min. in suspensions of $S_{41}$ containing $10^6$-$10^8$ cfu/ml prior to infection. Lower emergence percentage (60-80%) were observed using the antagonistic isolate $B_1$.

In case of potato common-scab disease, a complete control could be achieved by treating the soil with "Homai-80" at the rate of 2.5 g/kg . However, soil treated with higher doses of Homai-80 (5 g/kg) produced deformed plants with significantly lower tuber yield. Biological

control of common-scab disease through inoculation of infested soil with the antagonistic isolates $B_1$ or $S_{41}$ gave satisfactory results ; disease index percentages were about 6% and 10%, respectively, while disease index percentage of check treatment was about 20 % .

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## " الملخص العربـــى "

## المقاومه الكيماويه والبيولوجيـه لبعـض البكتريات الممرضه للنبات

أجرى هـذا البحث لدراسه المقاومه الكيماويه والبيولوجيه لبعض البكتيريات الممرضه للنبات وهى :

البكتيريا Agrobacterium tumefaciens المسببه لمرض التدرن التاجى ،

البكتيريا Erwinia atroseptica المسببه لمرض الساق السوداء فى البطاطس ،

البكتيريا Erwinia carotovora المسببه لمرض العفن الطـــرى ،

البكتيريا Streptomyces scabies المسببه لمرض الجرب العادى فى البطاطس .

بالنسبه للمقاومه الكيماويه فقد اجريت تجارب معمليه لدراسه التأثير المثبط لخمسه مبيـــدات فطريه وخمسه مبيدات حشائش ( تستخدم قبل الانبات ) على نمو البكتيريات الممرضه المذكـــوره . كانت المبيدات الفطريه المستخدمه هـى : بنليت ، هومای ـ ٨٠ ، روفرال ، تكتو ، فيتافاكـــس / كابتـــــان . أما مبيدات الحشائش المستخدمه فكانت : بلادكس ، اينايد ، ابتام ، لينـــورون ، ســـينكور . كما استخدم المضاد الحيوى " استريبتو مايسين " كمبيد بكتيرى قياسى بغرض المقارنه .

اوضحت نتائج الاختبارات المعمليه ان المبيد الفطرى " هومای ـ ٨٠ " يتفوق على المبيـــدات الاخرى المستخدمه حتى انه فاق فى تأثيره المثبط لنمـــو البكتيريا A. tumefaciens المضاد الحيوى ( الاستريبتو مايسين ) . فبتقدير أقل تركيز قاتـل للمبيد " هومای ـ ٨٠ " وجـــد انه يقع بين ٢٥ ـ ٣٠ جزء فى المليون فى حاله البكتيريا A. tumefaciens بينما وصـل هـذا التركيز الى ١٠٠ ـ ٢٠٠ جزء فى المليون من المضاد الحيوى ( استريبتومايسين ) . وفى حالـــه البكتيريا Str. scabies فقد كان اقل تركيز قاتل لكل من المبيد " هومای ـ ٨٠ " والمضاد الحيوى " استريبتومايسين " يقل عن ١ جـزء فى المليون . أما فى حاله كلا من البكتيريا E. atroseptica ، البكتيريا E. carotovora فقد كان اقل تركيز قاتل لهما مـــن المبيد " هـومای ـ ٨٠ " يقع بين ٢٠ ـ ٢٥ جزء فى المليون . بينما كان اقل تركيز قاتل لهما من المضاد الحيوى " استريبتومايسين " يقل عن ١ جزء فى المليـــون .

بالنسبه للمقاومه الحيويه فان عمليات العزل التى استخدم فيها مئـات من عينات التربه الـــتى جمعت من حقول ومناطق مختلفه اسفرت عن الحصول على ٣١ عزله تظهر تضادا على الاطبـــاق للبكتيريات الممرضه المذكوره ، وكانت ٢٨ من هـذه العزلات تتبع البكتيريات المتجرثمه ذات الشكل العصوى والاربعه عزلات الاخرى كانت تتبع البكتيريات الخيطيه ( الاكتينوميسيتات ) ، وقد تميـــزت العزلات $B_1$ ، $B_{43}$ ، $S_{41}$ بكفاءه أعلى من حيث التضاد مع البكتيريات الممرضه عن باقى العزلات الاخرى . وقد تم اجراء اختبارات التعريف لهذه العزلات اعتمادا على الصفات الشكليه والمزرعيه والبيوكيماويه ووجـد ان العزله $B_1$ تشبه فى صفاتها البكتيريا Bacillus megaterium بينما اظهرت العزله $B_{43}$ تشابها فى صفاتها مع البكتيريا B. brevis فى حين اظهرت صفات العزله $S_{41}$ انها تتبع الجنس Streptomyces .

ولتقييم النتائج المعمليه للمقاومه الكيماويه والبيولوجيه فقد اجريت تجارب اصص لاختبار كفاءه المبيد " هوماى ـ ٨٠" وكل من العزلات المضاده $B_1$ ، $B_{43}$ ، $S_{41}$ فى مقاومه امراض التدرن التاجى ،والساق السوداء فى البطاطس ،والعفن الطرى ،والجرب العادى فى البطاطس . وقد اوضحت النتائج المتحصل عليها ما يلى :ـ

فى حاله مرض التدرن التاجى فأن غمس الجزء السفلى من بادرات عباد الشمس لمدة ٥ دقائق فى محلول مائى تركيزه ٠ر٢٥٪ أو ٠ر٥٠٪ من " هوماى ـ ٨٠" قبل احداث العدوى الصناعيه بالبكتيريا A. tumefaciens قد أدى الى تثبيط تكوين التدرنات بنسبه حوالى ٧٦٪، ٨٣٪ على الترتيب .بينما نتج عن غمس الجزء السفلى من الشتلات لمدة ٥ دقائق فى معلق يحتوى على $10^{6}$ ـ $10^{8}$ خليه/ مل من العزله المضاده $B_1$ أو $B_{43}$ قبل اجراء العدوى الى تثبيط تكوين التدرنات بنسبه حوالى ٤٦٪ و ٦٥٪ على الترتيب .

فى حاله مرض الساق السوداء فى البطاطس فقد أدى غمس قطع التقاوى لمدة ٥ دقائق فى محلول مائى تركيزه ٠ر٢٥٪ أو ٠ر٥٠٪ من المبيد هوماى ـ ٨٠ قبل اجراء العدوى بالبكتيريا E. atroseptica الى زياده نسبه النباتات السليمه الناتجه من قطع التقاوى المعديه بنسبه ٨٠٪ ، ١٠٠٪ على الترتيب. بينما أدى غمس قطع التقاوى لمدة ٥ دقائق فى معلق يحتوى على $10^{6}$ ـ $10^{8}$ خليه /مل من العزله المضاده $S_{41}$ قبل العدوى الى زياده نسبه النباتات السليمه الى ١٠٠٪ ، غير انه فى حاله غمس قطع التقاوى فى معلق العزله المضاده $B_1$ كانت نسبه النباتات السليمه الناتجه اقل ( حوالى ٧٣٪ ) .

فى حاله مرض العفن الطرى فقد أمكن حمايه قطع التقاوى من التعفن كلية وذلك بغمسها لمدة ٥ دقائق فى محلول مائى تركيزه ٠ر٢٥٪ من المبيد " هوماى ـ ٨٠" قبل اجراء العدوى بالبكتيريا E. carotovora وكانت نسبه الانبات لقطع التقاوى ١٠٠٪ ، وقد امكن التوصل لنفس النتيجه بغمس قطع التقاوى لمدة ٥ دقائق فى معلق يحتوى على $10^{6}$ ـ $10^{8}$ خليه /مل من العزلة المضادة $S_{41}$ . غير أنه قد لوحظ أن نسبه الانبات كانت أقل فى حالة معاملة قطع التقاوى بالعزلة المضادة $B_1$ ( حوالى ٦٠ ـ ٨٠٪)

فى حاله مرض الجرب العادى فى البطاطس فقد أمكن تحقيق مقاومه كامله للمرض وذلك بمعامله التربة المحتويه على Str. scabies بالمبيد " هوماى ـ ٨٠" بتركيز ٥ر٢ جم/كجم تربه. ولكن النباتات الناتجه فى التربه المعامله بجرعات اعلى من المبيد ( ٥ جم/كجم تربه ) كانت أضعف فى نموها وأقل فى محصول الدرنات . بينما أدى تلقيح التربه بالعزلات المضاده $B_1$, $S_{41}$ الى انخفاض معنوى فى شدة الاصابه بالمرض حيث كانت شدة الاصابه ٦٪ ، ١٠٪ على الترتيب فى حين وصلت هذه النسبه الى حوالى ٢٠٪ فى معاملة المقارنه .

# المقاومة الكيماوية والبيولوجيه لبعض البكتيريات المسرضه للنبـــــات

رساله مقدمه مـن

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بكالوريوس فى أمراض النبـــات
( ١٩٧٩ )

للحصـول علـى
درجـة الماجستير فـى أمـراض النبـات

من قسم النبات الزراعى
بكلية الزراعـه ـ جامعة طنطـا

( ١٩٨٥ )