ANTIBIOTICS IN THE CONTROL OF CITRUS FRUIT ROTS

by

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A Thesis submitted

to the faculty of the

Department of Plant Pathology

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in the Graduate College, University of Arizona

1955

Approved: Alice M. Boyle, Jan. 19, 1955

Director of Thesis Date

ACKNOWLEDGMENT

The writer wishes to express his thanks to Dr. R. B. Streets and Dr. P. D. Keener for their interest and encouragement during the investigation, and for their helpful criticism of this manuscript. My sincerest gratitude is due Dr. Alice Boyle for her guidance and valuable advice throughout the entire course of the investigations here reported upon as well as in the preparation of the thesis. All the photographs used in this thesis were by Dr. Boyle. The fruits used in the research were kindly supplied by Dr. Streets.

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FOREWORD

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INTRODUCTION

Citrus has been grown in Mesopotamia since the time of Babylonia (2200 B.C.). The citrus tree was one of the favorable trees in Seven Heaven and Garden of Aden. The trees were grown as ornamentals, but during the Abbasid Period (750-1258 A.D.), the Golden Age in Mesopotamia, fruit trees and especially the citrus were planted in great numbers in many orchards. This was due to the fact that the prosperity of the country depended upon the agricultural income. There was a great deal of effort used in building dams, opening canals, establishing and plantations of crops over large areas of the valley. There was no starvation among the thirty million people at that time; in fact, the opposite was true. Grains and dates were exported to Persia, India, Syria, Arabia, and Egypt.

In 1285 A.D. the country was invaded by Hulaku Khan and his barbarian army, who destroyed the country in many aspects, including the agriculture. Trees were burnt or cut down; dams and canals were destroyed; many fields and large areas of the fertile valley were left bare and eroded.

Since that time up to 1918, the country was colonized by different nations: Fouris, Turkish, English, and German. As a matter of fact, many foreign governments did not care about rebuilding what was destroyed and improving what was present, except at the time of Mid'-hat Busha, a Turkish governor (1869-1872 A.D.), who really did his best to do something good for the country. He distributed the lands among the farmers, opened and cleaned, buried and destroyed canals, and he brought life to many deserted lands. After World War I, in 1921, Iraq got its independence. The new government of Iraq undertook rebuilding the nation not only in agriculture, but also in many other fields.

Dams, canals, and roads were built and small plots of land were given to individual farmers. Agricultural schools were established and financing of the growers was undertaken by the government. These things were done to improve the agricultural conditions in the country. Improvement showed first in areas where farmers owned their own land, where small sections were often planted to citrus. Because they depend entirely on the land for their living, these small land owners are willing to improve conditions of growing, picking, and shipping if the knowledge of how to bring about improvements is made available to them. In regions where large acreages of field crops are grown, the feudal system still exists. The farmers working under this system realize little return for their efforts. Since improvements in farming methods would not change their living conditions, they have no interest in learning new methods.

The map of Iraq (Plate I), shows the main citrus-growing regions labeled 1, 2, and 3. Ba quoba, Khalis, Mendili and Khanaqin Qadha of Dialah Liwa, 1, are the main producing areas. Next are Baghdad, Kadhmain Qadha of Baghdad Liwa, 2, and the last is Kerbelah Qadha of Kerbelah Liwa, 3. The citrus trees in these areas are grown under favorable conditions of soil, water, and climate.

There are a few villages in each Qadha (section of a county) of the already mentioned areas, 1, 2, and 3, Liwa (county). The fruits are brought by animals to the nearest railroad stations where they are kept often in the open, waiting for a car to be loaded. The railroad car is sent to the large cities in the country. The fruits are usually shipped by trucks from the villages to the scattered cities to which they are not joined by railroad or paved highways but by rough, unimproved roads. The trouble arises because of lack of knowledge of right principles in harvesting, handling, shipping, and storage, causing the farmers to often lose the market price.

Fruit rots and decay are due to injuries or abrasions to fruits in harvesting and handling. The lack of education and facilities of many growers forces them to use old customs which result in injuries that originate in numerous ways. Injuries occur when pulled fruits with long stems puncture other fruits, by dropping and throwing the fruits into sacks, by improper loading, and by contact with sand or gravel, and protruding nails left in field boxes.

Then, transportation is not always available to the growers for shipping their fruits to points where there are good market prices. Shipping is often delayed and the fruits may be left in the open under unfavorable temperature and moisture conditions. This leads to the development of fruit rots.

There are no refrigerator cars or trucks and no ventilation or moisture control. When the fruits are shipped by cars or railway or truck, it takes long periods of time to arrive. Most of the railways and their trucks were brought and used for military purposes and they are not suitable for fruit shipment. However, there is no other choice, except shipping by animals, (and that is worse), or by car resulting in jolting over rough roads. This takes more time for arrival. These conditions often cause mechanical injuries which lead to infection of the fruits.

The fruits, upon arrival in the market, are usually kept in damp, hot, non-ventilated and unclean places if they are not sold that day. If it happens that more fruits arrive that day or the next, the fruits may stay a few days under unfavorable conditions which lead to the development of the fruit rots.

REVIEW OF THE LITERATURE

In preparation for work on control of citrus fruit rots, a review of the literature, with two objectives in mind, was undertaken. First, it was necessary to have a knowledge and understanding of the diseases of citrus fruits and the organisms that cause them, and second, to learn the methods so far used to control these diseases.

Fawcett (7) classified the rot or decay of fruits into three general types:

1. Originating in injuries or abrasions to the rinds. Examples of type are:

   a. Blue contact mold rot due to *Penicillium italicum*.

   b. Common green mold rot due to *Penicillium digitatum*.

   c. Pink rot due to *Penicillium roseum*.

   d. Anthracnose spot due to *Colletotrichum gloeosporioides*.

   e. Black pit due to *Pseudomonas citriputeale*.

   f. Aspergillus rot due to *Aspergillus niger*. This fungus rots the fruit at high temperatures.

2. Originating without definite abrasions or mechanical injuries to

the rind. Examples of this type are:

a. Brown rot due to Phythiacystis citrophthora.

b. Botrytis or Gray mold due to Botrytis cinerea.

c. Cotton rot due to Sclerotinia libertiana.

d. Sour rot due to Oospora citri-aurantii.

3. Interior breakdown. Examples of this type are:

a. Alternaria rot (center rot) due to Alternaria.

4. Stem end rot. Examples of this type are:

a. Stem end rot (light, pliable, leathery) due to Phomopsis californica.

b. Stem end rot (dark, pliable, leathery) due to Diplodia natalensis.

Fawcett (7) and Lee (9) consider that the age and the condition of the fruit, injuries, humidity, and temperature are important contributing factors to the development of fruit rots or decay. Ramsey (27) believes that the disease of the fresh fruits caused by fungi during various phases of the marketing process are due either to contamination and infection that occur during the growing season or to infections through injuries incidental to harvesting, processing, packing, and transporting the produce. He also adds that more than 100 fungi may cause decay and blemishes in commercial types of fruits.

Nadel-Schiffman (24) found that fallen fruit in orchards on light soil was attacked more severely by Penicillium digitatum, while in heavy soil Penicillium italicum was more troublesome.

In this country many precautions are taken to avoid the great loss of fruits due to fungi and bacteria, causing citrus fruit rots. There are four general means of prevention that will be discussed here: mechanical, physical, chemical, and biological.

I. Mechanical

Fawcett (8) mentions two things that require emphasis above all else--picking the fruit when it is thoroughly dried after rains, fogs, or heavy dews, and careful handling of the fruit to avoid injuries during all operations from tree to market.

Powell (26) reported that the least decay has developed under all methods of shipment in sound, carefully handled oranges, and the greatest amount has developed in those that were mechanically injured.

II. Physical

Ramsey (27) finds the important factors in the control of blue and green mold are careful handling during picking and packing, packing house sanitation, pre-cooling and prompt storage at 30-32 F. In addition, Fawcett (8) mentions the use of hot water (115-120° F.) and soap, followed by drying and paper wrapping, not only for the purpose of cleaning the fruit, but also as a partial control of green and blue mold decays.

III. Chemical

In combatting citrus fruit rots, the most desirable and usually the least expensive means lie in the line of prevention rather than in that of treatment after the infection has started. Fawcett (8) mentions that in the prevention and treatment of disease two things must always be considered. These are the effectiveness of the methods and the expense of the treatment. Lauriol (21) concludes that complete control of Penicillium on citrus can be secured by chemical treatment. Borax at 5 percent in hot water for five minutes, judiciously applied, gives protection. There are many chemicals that have been tested and used on citrus fruits in packing houses, in shipment and in storage, but their superiority is based on their effectiveness or the fact that they are less costly, less poisonous, and less disagreeable.

Fulton and Bowman (13) found that soaking the fruits in 5 percent Sodium borate (Borax) or Boric acid solution for five minutes, then drying and storing at 70° F., 90 percent relative humidity does a great deal to decrease the decay of fruits in storage and transit. Barger and Hawkins (3) consider Borax as effective as Boric acid in control of blue and green mold decay and is much cheaper. Fohson (10) reported that Borax-treated fruit is especially apt to wilt; therefore, it must be waxed after the treatment. In Florida, the use of Sodium-ortho-phenyl-phenate has advantages over Borax for the treatment of citrus fruits. It acts quickly and can check decay in citrus even when the application is delayed until several days after the harvest. The treatment is commonly applied by dipping the fruit either momentarily or for about two minutes in a tank containing a 1-1.25 percent concentration of the chemical. It must be followed by a fresh water rinse and drying. In California, the use of Sodium carbonate (soda ash) 1.25-2 percent is preferred to Borax for use on lemons only.

Hopkins and Louck (18) reported that an excellent control in Hamlin, Parson-Brown, Pineapple and Valencia orange was achieved when the fruits were immersed in Dowicide A (1.5-2 percent) plus Hexamine

(0.75-1 percent) for one to two minutes at 80-100° F. When extensive laboratory experiments were carried out on the control of citrus molds with various compounds in comparison with Borax, Perret and Lespes (25) found that the best control was obtained by soaking the fruits for five minutes in cold 5 percent Thiourea on the day of harvesting or two days later. Mold can be reduced according to Fulton and Bowman (11) on citrus fruits by using 3 percent Sodium bi-sulfate following soap and water wash and rinsing them, spraying with fresh water to remove excess solution, and finally drying before grading, sizing and packing. The continued advances in chemical treatment of fruits doubtless will bring new and better compounds.

IV. Biological

Fawcett (9) mentioned a method of prevention which may have an interesting future--that of biological control. This involves the use of one organism to combat another. One organism may prevent the attack of a second by competition, inhibition of growth, or actual parasitism.

Gottlieb and Anderson (17) mention the great importance of antibiotics in combatting diseases of man, and discuss factors that have delayed exploring the possibility of using those agents against plant diseases. There is some experimental evidence accumulated according to these authors that gives hope of controlling some plant diseases with antibiotics. Examples of antibiotics found to be effective against plant diseases are: Antimycin, effective in controlling both apple scab caused by *Venturia inaequalis* and fire blight caused by *Erwinia amylovora*. Goodman and Ark (15) indicated very good control of fire blight with Agrimycin-100 (contains 100 ppm. of Streptomycin and 10 ppm.

of Terramycin) in a three-blossom spray schedule. Boyle (4) used Penicillin against Erwinia carnegieana which causes bacterial necrosis of Giant Cactus. Zaumeyer, Mitchell, and Preston (30) proved that Streptomycin sulfate, applied in time, can cure bean plants after infection with the halo blight bacterium, Pseudomonas phaseolicola. Dye (6) found that Streptomycin significantly retarded gall development and killed numerous galls on plants infected with Crown Gall caused by Agrobacterium tumefaciens. Riker, Gergary, Allen and Petersen (28) reported that 30 percent of 2452 streptomycetes produced antibiotics effective against one or more of the three species of Pythium arrhenomanes, Pythium ultimum and Rhizoctonia solani. Gopalkrishman and Jump (16) using Thiolutin (antibiotic) at 10 ppm. inhibited Pythium debaryanum, Sphaeropsis ulmicola and Sclerotinia fructicola, and at 50-100 ppm. inhibited Colletotrichum gossypii, Phomopsis (Diaporthe) citri, and Rhizoctonia solani. They found also that Thiolutin at 1 percent decreased incidence of infection in oats by Helminthosporium sativum from 53 to 3 percent. And, similarly, dusting cotton seeds with one-half to two percent Thiolutin resulted in a reduction of anthracnose caused by Colletotrichum gossypii of cotton while Katznelson and Sutton (19) found that Aureomycin and Polymyxin the most effective against Xanthomonas and Terramycin and Streptomycin against Pseudomanas, and that Neomycin and Chloramphenicol were effective against Corynebacterium.

The use of antibiotics, not only in combatting diseases, but also in food preservation, offers interesting possibilities (20). As adjuncts in the sterilization of foods by heat; to prevent the build-up of bacterial contaminations prior to organic processing, for example, in the preparation of fruits and vegetables for freezing; delaying the deterioration of perishable food during transportation and marketing; as an adjunct to refrigeration, thus delaying the onset of spoilage in fresh foods; prevention of the growth of food poisoning organisms in certain processed foods; controlling contamination in biological processes such as fermentation.

Godkin and Cathcart (14) found that subtilin controlled both food poisoning organisms and the general heat-resistant spoilage flora.

Morse (23) has shown that the combination of subtilin at 20 ppm. and boiling for ten minutes has resulted in successfully canning vegetables such as peas, corn, asparagus, cauliflower, beans and mushrooms. Food packs thus sterilized remained unspoiled for a period as long as eight months. Smith (29) has recently reported that the experimental use of Streptomycin aided in the reduction of bacterial soft-rot in packaged spinach. He found that an additional day of shelf life at room temperature could be obtained by spraying or dipping spinach in a solution of 0.05 to 0.1 percent Streptomycin.

In Iraq, nothing has been done yet to avoid the occurrence of such troublesome fruit rots, although many owners have been losing high percentages of their crop. Since I became interested in plant pathology, I have wished to learn and understand the principles of that science with the idea of rendering humble service to the people of my country.

Since antibiotics have been found to inhibit some fungi that cause plant diseases and are also found to be useful in the preservation of foods, we undertook the testing of certain of the antibiotics against some fungi responsible for the rotting of citrus fruits.

MATERIALS AND METHODS

In the work reported in this paper, four fungi causing citrus fruit rots were used: Penicillium italicum, Penicillium digitatum, Aspergillus niger and Sclerotinia libertiana. These were chosen because they are perhaps most common as causes of citrus fruit rots and because cultures were readily available.

Two series of antibiotics were used in the experiments.

I. Water dilution of the following:

a. Pleocidin

b. Neomycin

c. Magnomycin Hcl

d. Tetracycline

e. Bacitracin

f. Terramycin Hcl

II. Sensitivity disks of the following:

a. Chloromycetin (10, 30, 60 mcg.)

b. Aureomycin (10, 30, 60 mcg.)

c. Terramycin Hcl (10, 30, 60 mcg.)

d. Polymxin (5, 10, 30 mcg.)

e. Bacitracin (2, 10, 20 units)

The former were supplied by Merck and Company, Charles Pfizer and Company, and Mathieson Chemical Corporation; and the latter by Difco Laboratories.

In the first experiment the antibiotics in the first series were tested against the four fungi on citrus fruits. Mature Valencia oranges were divided into groups of five and each five was sprayed with a solution of an antibiotic at each of three concentrations (100, 250, 500 ppm. or 10, 25, 50 mg. to 100 cc. sterilized distilled water.).

Spraying with the antibiotic was followed by inoculation of the fruits with one of the four fungi. A spore suspension was prepared in sterile distilled water in the case of the first three fungi. This inoculum was sprayed generously on the fruits. To inoculate with Sclerotinia libertiana, sclerotia were taken from agar slants and placed upon the fruits. Suitable controls were provided as follows: one set of fruits sprayed with the antibiotic and uninoculated in order to determine whether or not injury to the fruit resulted; one set of fruits was left untreated with antibiotics and inoculated; a third group was untreated and uninoculated. All groups of fruits were stored in the same way by placing each set of five in a plastic dish with the cover loosely put on to provide ventilation. The fruits were kept at room temperature.

In the second experiment the antibiotics were tested against the four fungi on agar plates. In one series lima bean agar at pH 7 was prepared; at 50° F it was poured into Petri dishes which were amply covered by 12 cc. of agar and later inoculated with pure cultures of the fungi. A bit of the growth from cultures of the first three fungi was placed on agar plates at four points in a square-shaped area. To inoculate with Sclerotinia libertiana, sclerotia were taken from agar slants and placed in the same manner. The fungi were allowed to grow for a day. Solutions of the first series of antibiotics at 500 ppm. were prepared, and Peni-cylinders were placed in the middle of the plate and filled with the antibiotic to be tested. Controls were left without antibiotic. This experiment was repeated to check results.

In the third experiment, three concentrations (100, 250, 500 ppm.) of Pleocidin were prepared and used against the four fungi to determine which one of the three concentrations was more effective against every one of the four fungi. In one set the agar plates were inoculated with the fungus by cross streaking. Paper disks soaked in solution of a certain concentration were placed at the crossing point of the streaks. Each of the four fungi was treated in this way with each of the three concentrations of antibiotic. One set of plates, with the fungi but with no antibiotic added, served as controls. One duplication for this experiment was made.

In the fourth experiment the antibiotics in the second series (Sensitivity disks) were tested against the four fungi on agar plates. Inoculation was made as in the second experiment. The antibiotic was applied at three concentrations for each fungus. The plates were kept as in the other experiments at room temperature, about 72° F.

RESULTS

The results of the use of Terramycin in experiment one are given in Table I. Like results were obtained in the use of Neomycin, Magnomycin Hcl, Tetracycline, Bacitracin, and Terramycin Hcl. The experiment indicated that the fruit treated with antibiotics and uninoculated with the fungi in the first set showed an injury to the fruits in 15-20 days due to the antibiotics, especially with the concentrations of 500 ppm. In the second set in this experiment, the fruits inoculated with the fungi showed in the same period of time symptoms typical of the rot caused by the particular fungus used. There was only one exception. The fruits treated with Pleocidin developed no disease symptoms at any concentration of antibiotic used. They did, in 25 days, all become wrinkled due to water loss. This condition was to be expected. All of the fruits in this series were in the same condition at the completion of the experiment whether they were treated with antibiotic and inoculated, treated with antibiotic only, or left untreated and uninoculated.

The results of experiment two, in which concentrations of antibiotics at 500 ppm. were used, are shown in Table II. Neomycin, Magnomycin Hcl, Tetracyclin, Bacitracin, and Terramycin Hcl showed no positive results. Two duplications were made, but Pleocidin was the only antibiotic used that inhibited the fungi.

The results of the use of Pleocidin in experiment three are shown in Table III. This table shows that the antibiotic inhibited all the fungi most effectively at a concentration of 250 ppm. Plates I, II, III and IV show the inhibition by Pleocidin on the four fungi.

The results of the use of the second series of antibiotics (the sensitivity disks) in experiment four when tested against the four fungi on agar plate were negative.

TABLE I - Result of the use of Terramycin Hcl on fruits

Concentration ppm.	Fungus	Inhibition 15-25 days	Symptoms
100	*Penicillium*	-	The fruits showed water-soaked and soft areas which enlarged slowly, then white soft mycelium developed on the surface followed by the development of the blue spore masses. The blue color spread and left only a very narrow white fringe. The white mycelium was powdery and deep-seated.
250	*italicum*	-	
500		-	
100	*Penicillium*	-	The fruits showed water-soaked soft areas which enlarged rapidly and a white mycelium appeared rapidly. Olive-green spores formed a dust cloud when distributed. The white area of mycelium was pasty and wrinkled.
250	*digitatum*	-	
500		-	
100	*Sclerotinia*	-	The fruits were covered with a white cottony or fluffy growth of mycelium, which later formed cheesy, irregular, black
250	*libertiana*	-	
500		-	

TABLE I - Result of the use of Terramycin Hcl on fruits (cont'd)

Concentration ppm.	Fungus	Inhibition 15-25 days	Symptoms
			sclerotia. The softening of the fruit was slow and the skin became leathery.
100	*Aspergillus niger*	-	The fruits became soft and light in color; the tissue became wrinkled. The decayed area was sunken and covered with the mycelium which formed flaked powdery layers which contained numerous spores.
250		-	
500		-	

TABLE II - Result of the use of Pleocidin - 500 ppm. in culture

Fungus	Inhibition zone in mm.	Fungus	Inhibition zone in mm.
Penicillium italicum	9	Aspergillus niger	6
Penicillium digitatum	10	Sclerotinia libertiana	11

TABLE III - Results of the use of various concentrations of Pleocidin in culture

Concentration in ppm.	Fungus	Inhibition zone in mm.
100	Penicillium italicum	6
250		12
500		10
100	Penicillium digitatum	4
250		14
500		11
100	Sclerotinia libertiana	7
250		16
500		12
100	Aspergillus niger	5
250		15
500		7

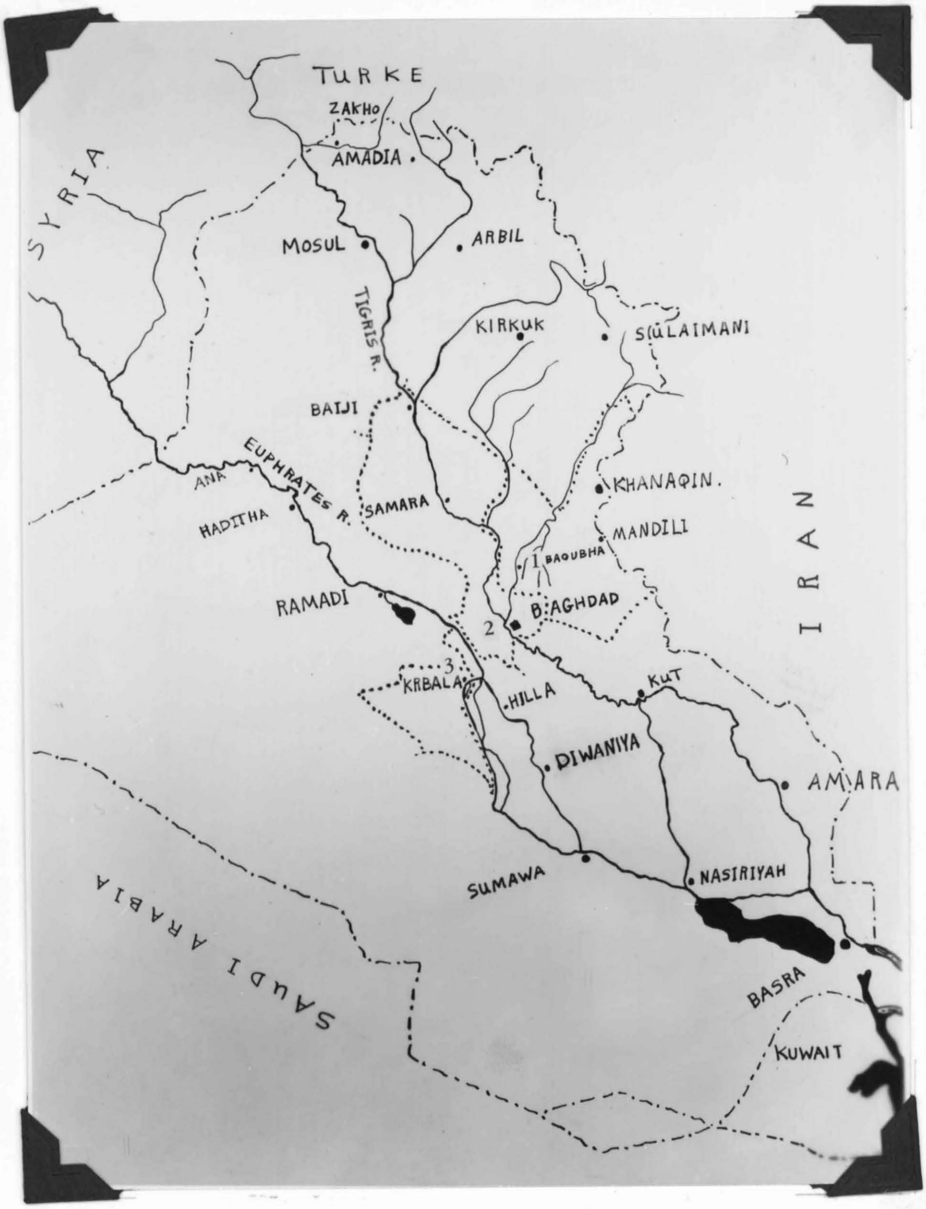

PLATE I. Map of Iraq, showing main citrus-growing areas indicated as 1, 2 and 3; and the main cities to which citrus is shipped.

PLATE II. Result of experiment three, showing inhibition by Pleocidin, 500 ppm., on Penicillium italicum. A, control plate without addition of the antibiotic; B, shows zone of inhibition around antibiotic-soaked paper disk.

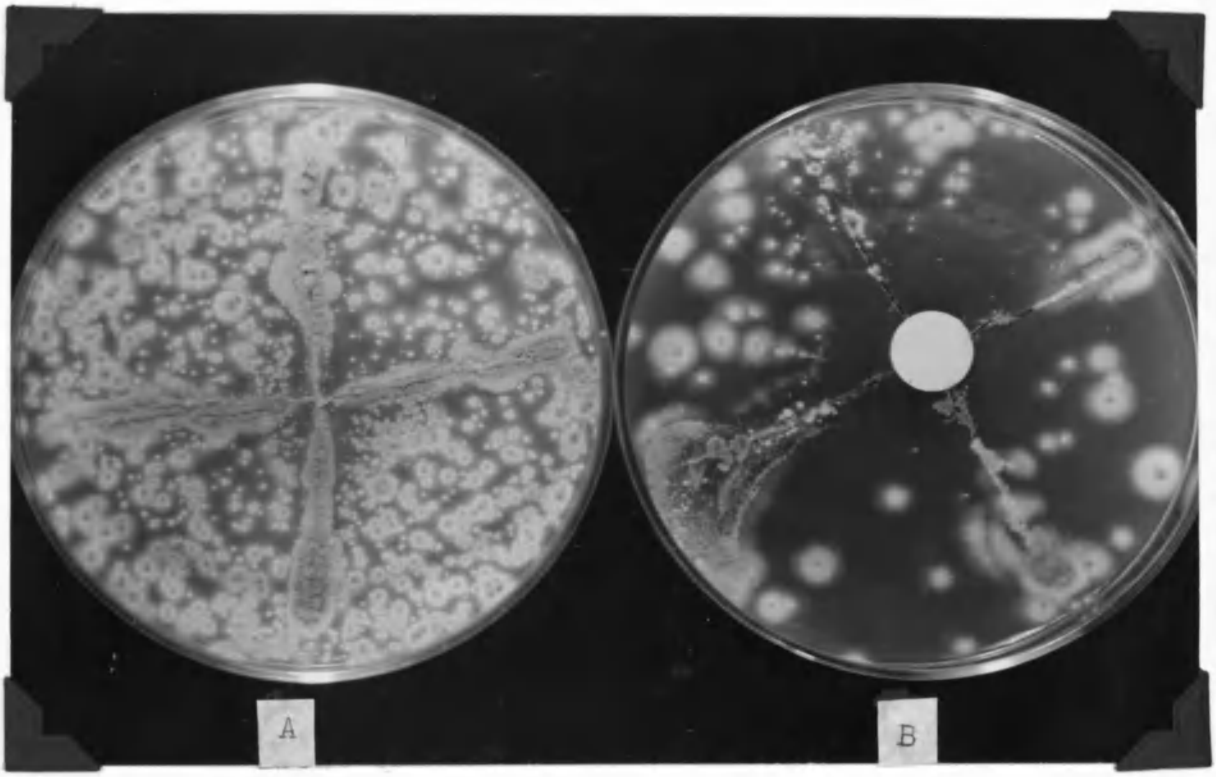

PLATE III. Result of experiment three, showing inhibition by Pleocidin, 500 ppm., on *Penicillium digitatum*. A, control; B, effect of antibiotic.

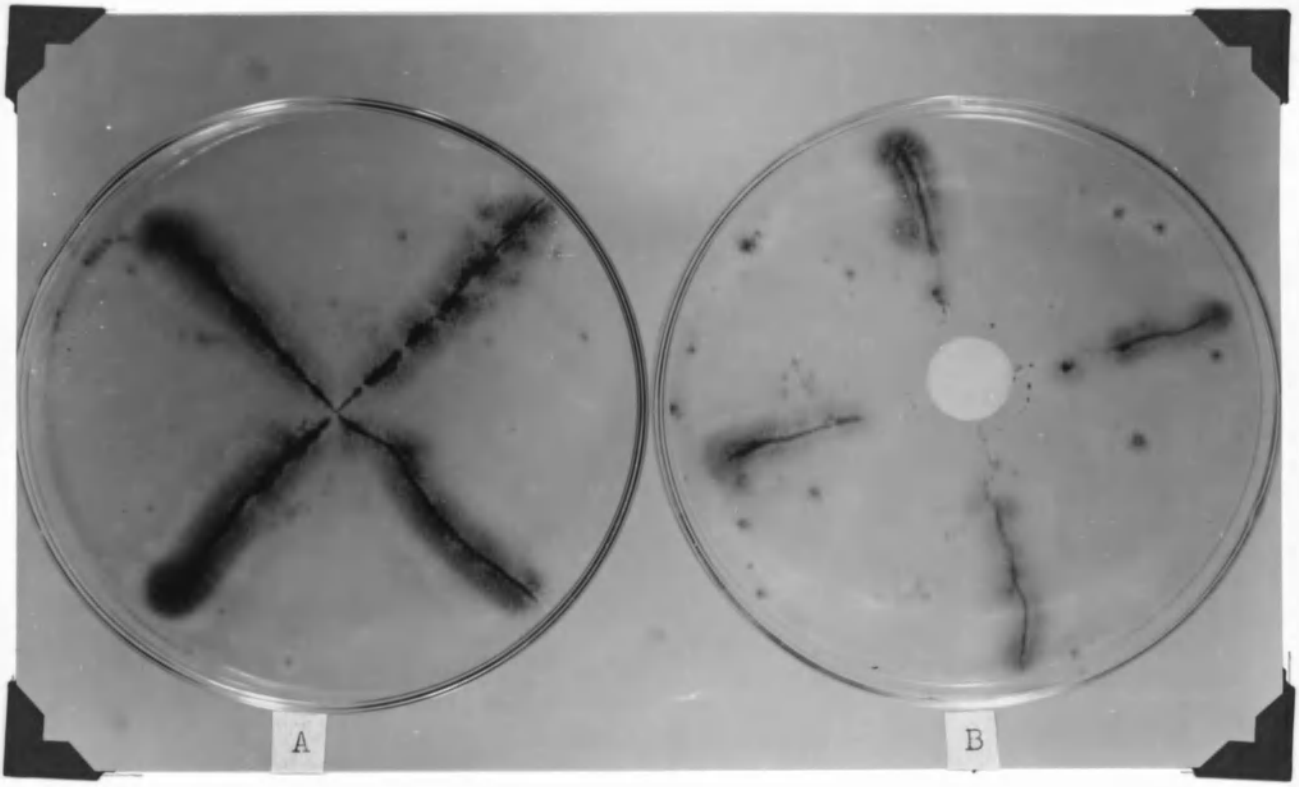

PLATE IV. Result of experiment three showing inhibition by Pleocidin, 500 ppm., on *Aspergillus niger*. A, control; B, effect of antibiotic.

PLATE V. Result of experiment three, showing inhibition by Pleocidin, 500 ppm., on *Sclerotinia libertiana*. A, control; B, effect of antibiotic.

CONCLUSION AND DISCUSSION

The work reported upon in this paper shows that the antibiotic, Pleocidin, at 250 ppm., not only inhibits the growth of Penicillium italicum, Penicillium digitatum, Aspergillus niger and Sclerotinia libertiana in culture but prevents the rot of fruits by these four fungi. This discovery gives hope that some such treatment might be practical for use in my country, Iraq, to protect citrus fruits from rots and delay their deterioration under the very primitive conditions of harvesting, transporting, and marketing that exist in that country.

Pleocidin (5) is produced by isolant #272, which resembles Streptomyces lavendulae, when grown on any of several media. It was found that the purification of Pleocidin was difficult but was accomplished as described by Charney, Roberts, and Fisher (5). The purified Pleocidin hydrochloride is a white hygroscopic powder soluble in water, methanol and ethanol to yield colorless solutions. The antibiotic is most stable in aqueous solution in the region of pH4 to 6. Charney, Roberts, and Fisher (5) report the antibiotic as having an inhibiting effect on Aspergillus niger, Alternaria mali and Saccharomyces cerevisiae, as well as on certain bacteria.

The possibilities are extensive for further work on the problem of citrus fruit rots and their control by antibiotics. If there had been time for the testing of other antibiotics against citrus fruit rotting fungi, undoubtedly others showing inhibitory properties would have been discovered. Perhaps some of these might prove of more value in solving the problem of citrus rots than would Pleocidin.

Another interesting study that might prove of great value and which the writer would have liked to have undertaken had time permitted is the investigation of antagonistic organisms in soils of my country. Majunder and Sen (21) report interesting methods and results of such work in India.

Summary

Citrus fruit rots are troublesome in shipping, marketing, and storage in my country, Iraq. I undertook the problem reported on in this paper to understand the causal organisms and to find, if possible, a means of control of the diseases they cause.

The literature was reviewed to gain a knowledge of methods for protection and prevention used in the past.

The use of antibiotics in plant disease control and food preservation has proved successful. Therefore I tested a number of antibiotics on fresh fruits as well as on agar plates in an effort to find one that would prevent fruit rots and give the treated fruits longer shelf life.

As a result of my experiments, it is evident that Pleocidin at a concentration of 250 ppm. shows promise as a spray or solution to be used for the control of certain citrus fruit rots.

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