

## OBSERVATIONS ON THE INHIBITORY ACTIVITY OF A SPECIES OF BACTERIUM ON SOME FUNGI PARASITIC ON THE COCONUT PALM

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### INTRODUCTION

A chemotherapeutic approach to the control of plant diseases is rapidly gaining recognition in plant pathology. Although the use of antibiotic substances in controlling human and animal diseases has been one of the most outstanding medical developments in recent years, their use in controlling bacterial or fungal diseases of plants has hitherto been far less closely studied. There have, nevertheless been quite a number of instances pointing to the possibilities in this direction both by the direct application of specific antibiotics as well as by stimulating through appropriate manuring the micro-organisms naturally present in the soil to produce antibiotic

substances. (E. W. Russel, 1950, E. Grossbard, 1951). However, there appears to be very little published information on the value of antibiotics in affording protection to perennial plants against disease attack. Weindling has claimed (R. Weindling 1932, 1934, 1936) that the soil fungus *Trichoderma lignorum* can control the damping off of citrus seedlings caused by a species of *Rhizoctonia*. Stoddard and Dimond have shown (E. M. Stoddard and A. E. Dimond, 1951) that experimental chemotherapeutants "1182" and "1207" are effective in the control of *Fusarium* wilts of carnations and tomatoes and the virus disease of peaches. Successful results have been reported in preliminary trials with the soil application of a new systemic insecticide named Hanane, (C.R. 409) against mealy bug vectors of the swollen shoot virus of cacao (Anon 1951). Meiffren reports (Meiffren 1951) trials made with the organic fungicide cryptonal (neutral ortho-oxy-quinoline sulphate) on coconuts against attack by the fungus *Fusarium oxysporum*. Recent investigations

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have shown that a variety of anti-fungal substances such as gliotoxin and viridin isolated from the green mould *Trichoderma*, spp. gladiolic acid from *Penicillium gladioli*, musarin from an actinomycete, and antimycin from a species of *Streptomyces* have been used with varying degrees of success in plant protection (Grossbard, loc. cit). Experiments carried out in Britain have shown that a substance known as griseofulvin acts also as a systemic fungicide being taken up by the roots and translocated throughout the plant, thus protecting it against infection (P. W. Brian 1951). In the U. S. A. antimycin has been tested extensively in greenhouse trials and promises to be a useful antibiotic fungicide. It has been found to inhibit the germination and growth of the fungus *Venturia inaequalis* which is responsible for the scab disease of pomaceous fruit trees. These instances serve to show that, in the not too distant future the use of antibiotic substances might become popular in controlling parasitic plant diseases. We have been concerned in this Research Station with investigations on the diseases of the coconut palm and have shown that certain fungi like *Helminthosporium halodes*, *Botryodiplodia theobromae*, *Rhizoctonia bataticola*, *R. Solani* etc. are closely associated with the leaf and root diseases of the coconut palm in Travancore-Cochin. (K.P.V. Menon and Co-workers 1951). During

these investigations a species of bacterium was obtained which grew freely in common laboratory culture media and which when used against some of the above mentioned fungi afforded the plant tissue, some measure of protection from infection by them. We present in this paper results of our preliminary experiments with a strain of bacterial organism which was seen to exert antibiotic action over some fungi parasitic on coconuts.

#### EXPERIMENTAL

*Isolation of the bacterium:* After a series of trials using common laboratory bacteriological media it was found that a three per cent aqueous solution of "Bovril" autoclaved at 15 lb. pressure for 20 minutes was a suitable medium for the growth of the bacterium. Both in this culture solution and on Bovril agar the organism grew well and when examined under the microscope appeared as typical cocobacillary forms (See page 429 of Jordan Burrow's "Text book of Bacteriology"). It was aerobic and spore forming and appeared to be related to the *Bacillus anthracis* (*B. subtilis* group). It may be pointed out in this connection that *B. subtilis* has already been shown to possess antifungal properties (cited in R. A. M. 1952; 37 52., R. S. Vasudeva and co-workers 1952; H. D. Michener and N. Snell 1949; R. K. S. Wood 1951 and F. J. Newhook 1951).

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*Demonstration of the antifungal property of the bacterium:* The various fungi were inoculated into sterile petridishes containing appropriate culture media. The inoculum was planted in different dishes in different positions *viz.*, at the centre of the dish, at three spots to form the corners of an equilateral triangle with its centre at the centre of the petri dish and also in other geometrical positions to give different patterns. After the fungi had established themselves and commenced to grow, the petridishes were streaked with a 24-hour old culture of the bacterium in positions about 2 cms. away from the points of inoculation of the fungi. Thus the spreading fungal colony and the spreading bacterial colony could meet and exert their influences on each other. Among the different fungi isolated from diseased coconuts the following were tested in these trials.

- (1) *Helminthosporium halodes*,
- (2) *Botryodiplodia theobromae*,
- (3) *Rhizoctonia bataticola*,
- (4) *R. solani*,
- (5) *Gliocladium roseum* and a species of *Fusarium*.

The composition of the different culture media used in these trials are given below.

1 *Brown's Synthetic medium*

|               |        |
|---------------|--------|
| Glucose       | 2 gms. |
| Asparagin     | 2 "    |
| Potato starch | 10 "   |

|                    |          |
|--------------------|----------|
| Pot. phosphate     | 1.25 gm. |
| Magnesium sulphate | 0.75 gm. |
| Agar               | 20 gms.  |
| Distilled water    | 1000 cc. |

2 *Modified Brown's Synthetic medium*

|                        |           |
|------------------------|-----------|
| Glucose                | 2 gms.    |
| Potato starch          | 25 gms.   |
| Sodium nitrate         | 2 "       |
| Tripotassium phosphate | 1.25 gms. |
| Magnesium sulphate     | 0.75 gm.  |
| Agar                   | 20 gms.   |
| Distilled water        | 1000 cc.  |

3 *Richard's medium*

|                                 |          |
|---------------------------------|----------|
| Cane sugar                      | 50 gms.  |
| Potassium nitrate               | 10 gms.  |
| Potassium di hydrogen phosphate | 5 gms.   |
| Ferric chloride                 | a trace. |
| Magnesium sulphate              | 2.5 gms. |
| Agar                            | 20 gms.  |
| Distilled water                 | 1000 cc. |

4 *Nutrient agar*

|                 |          |
|-----------------|----------|
| Beef extract    | 3 gms.   |
| Peptone         | 10 gms.  |
| Agar            | 20 gms.  |
| Distilled water | 1000 cc. |

5 *Nutrient dextrose agar*

|                 |          |
|-----------------|----------|
| Beef extract    | 3 gms.   |
| Peptone         | 10 gms.  |
| Dextrose        | 10 gms.  |
| Agar            | 20 gms.  |
| Distilled water | 1000 cc. |

6 *Modified Czapek's medium*

|                                 |         |
|---------------------------------|---------|
| Sodium nitrate                  | 3 gms.  |
| Potassium di hydrogen phosphate | 1 gm.   |
| Magnesium sulphate              | 0.5 gm. |

|                    |           |
|--------------------|-----------|
| Potassium chloride | 0.5 gm.   |
| Ferrous sulphate   | 0.005 gm. |
| Zinc sulphate      | 0.005 gm. |
| Saccharose         | 5.0 gms.  |
| Agar               | 20 gms.   |
| Distilled water    | 1000 cc.  |

7 *Glucose asparagin medium*

|                                 |          |
|---------------------------------|----------|
| Glucose                         | 30 gms.  |
| Asparagin                       | 1 gm.    |
| Magnesium sulphate              | 0.5 gm.  |
| Potassium di hydrogen phosphate | 1.5 gms. |
| Agar                            | 20 gms.  |
| Distilled water                 | 1000 cc. |

8 *Bovril medium*

|                 |          |
|-----------------|----------|
| Bovril          | 30 gms.  |
| Agar            | 20 gms.  |
| Distilled water | 1000 cc. |

In all the trials made with different fungi growing on different media it was seen that the bacterium was able to inhibit the growth of the fungus. A typical instance is illustrated in Fig. 1 which shows how the bacterial strain was able to exert strong inhibitory action on the fungi *Helminthosporium halodes* and *Rhizoctonia bataticola*. In order to study the effect of the age of the fungal culture on the inhibitory action of the bacterium, streaking with the bacterium was made in petridishes containing fungi in different ages of growth. The inhibition of the fungus was apparent in most cases from about the third day. Fungal cultures up to five days old were strongly lysed by the

bacterium. The process was less vigorous in established and old fungal cultures. In the early stages of growth of the fungus the lytic action was quick and progressive. Even in old fungal cultures there was a short clearance in the petridishes round about the spots where bacterial streaks were made.

*Germination tests:* The effect of the bacterium on the germination of fungal spores was next studied. The fungus *Helminthosporium halodes* was chosen for this, since it forms spores freely. Hanging drop cultures were made in van Tieghem cells with the fungal spores alone and also with fungal spores and the bacterium mixed together in the drop. After an incubation period of twelve hours at laboratory temperature the hanging drops were examined under the microscope. It was observed that in the drops containing only the fungal spores there was excellent germination and profuse growth of the germ tubes. When bacteria were added to the spores of the fungus, germination of the fungal spores was arrested. A few scattered spores had formed vesicles from germ pores at either end of the spores. Further growth and ramification of the germ tubes was not observed. These tests were carried out with the spore suspension in sterile water and in different appropriate media. When the bacterium was added to the hanging

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drop there was inhibition of germination of the fungal spores.

*Inhibition experiments.* These experiments were carried out on liquid media (i) in test tubes of ordinary size (ii) in bigger sized test tubes and (iii) in small 100 cc. Erlenmeyer flasks, so that different surface areas of the fungus would be available. The medium used was Brown's synthetic medium without agar—10 ccs of this medium was used for small test tubes, 30 ccs for each of the bigger test tubes and 25 ccs. for each of the small flasks. The containers with the medium were sterilised as usual, inoculated with a spore suspension of *H. halodes* and incubated at laboratory temperature. The fungus grew as a black surface scum and when good growth was established 2 ccs. of the bacterial suspension was added to the small test tubes, 5 ccs. of the suspension to the bigger test tubes and 10 ccs. to the flasks. Controls were maintained in all cases where appropriate quantities of sterile distilled water were added instead of the bacterial suspension. In the containers to which the bacterial suspension was added, further growth of the fungus was arrested. The mycelial mat of the fungus formed on the surface prior to addition of the bacterial suspension soon started dissolving and in about 72 hours disappeared, due probably to the

lytic action of the bacterium. From these tubes it was then not possible further, to subculture the original fungus. The action of the bacterium was thus not only fungistatic but also fungicidal. Fig. II gives an illustration of the inhibitory action of the bacterium against *H. halodes* in an experiment carried out in large sized test tubes.

*Experiments on coconut leaves:* For the bacterial strain to be of any practical use in the control of the diseases of the coconut palm it should afford protection to coconut tissues from infection by parasitic fungi. To investigate this, it was decided to inoculate coconut leaves with the leaf rot organism *Helminthosporium halodes* and to find out whether in association with the bacterial culture, the fungus would exhibit the same amount of parasitic vigour. Small pieces, 3" long, of tender leaflets of coconut trees were placed in moist petridishes and inoculated with drops of spore suspension of the fungus. In a parallel series of petridishes, the inoculation drop consisted of a mixture of the fungal spores and bacterium. The dishes were maintained at laboratory temperature. Rotting of leaf tissue was observed in about 12 hours when the fungal suspension alone was used in the infection drop. When the mixture of the fungal spore and bacterium was used for

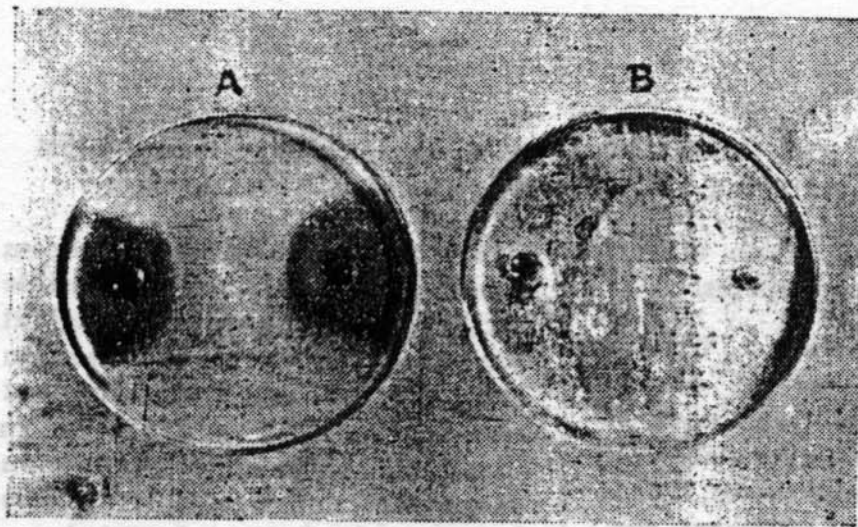


Fig: I  
 Inhibitory action of the bacterial strain on (A) *Helminthosporium halodes*  
 and (B) *Rhizoctonia bataticola*

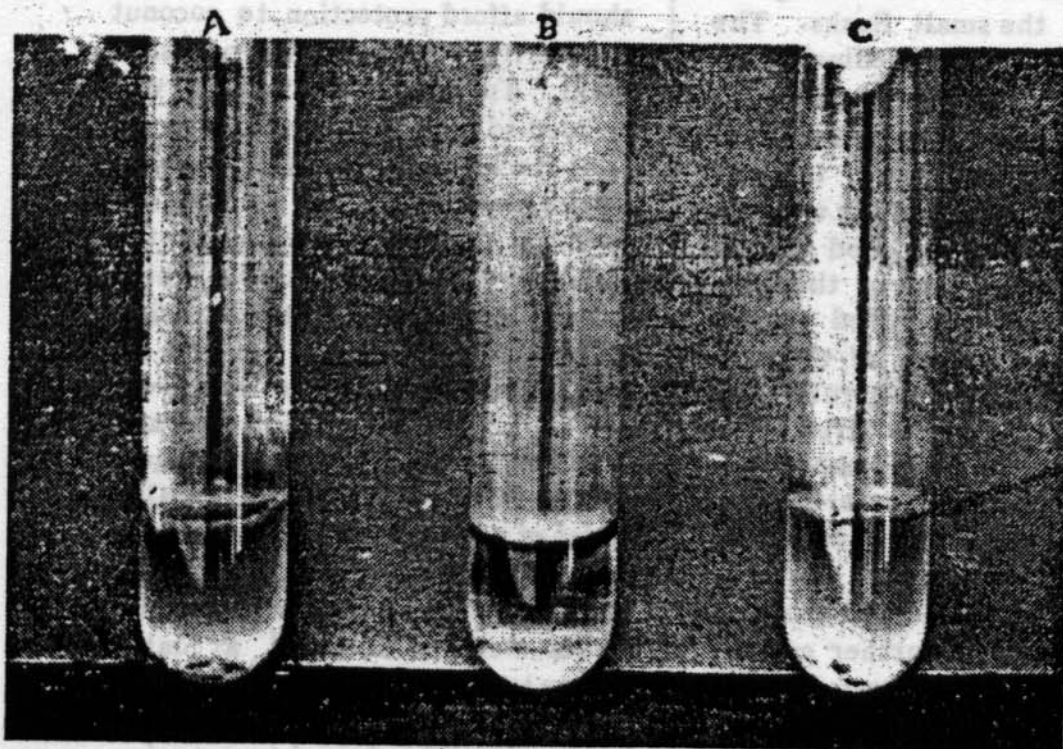


Fig: II  
 Lytic action of the bacterial strain on a liquid culture of  
*Helminthosporium halodes*.  
 A. Culture + 5cc. of the bacterial suspension  
 B. Culture + 5cc. of sterile water. (Control)  
 C. Culture + 10cc. of bacterial suspension

inoculation no infection was noticed, the bacterium apparently affording some sort of protection to the leaf tissue against fungal invasion. Similar experiments were also conducted, where coconut leaflets about 9-12" in length were sprayed with the spore suspension by means of a Devilblis atomiser and maintained in humid conditions under bell jars. Similar parallel sets were maintained, where the inoculum to be sprayed was a suspension mixture containing the fungal spores and the bacterium. In all cases the inoculated leaf tissue was incubated at laboratory temperature. In these experiments also it was seen that the presence of the bacterium in the inoculum afforded protection to the coconut leaf tissue against infection by the leaf-rotting fungi.

*Field experiments:* Three to five year old healthy coconut seedlings were selected for these trials. The tender shoots of the seedlings were inoculated as usual with a spore suspension of the fungus *Helminthosporium halodes* and a parallel series of inoculations were done where the inoculum was a mixed suspension of the bacterium and spores of the fungus. Controls were also maintained using sterile water and the bacterial suspension alone as inocula. Pieces of sterile cotton wool were soaked with the different kinds of inocula and they were introduced between leaflets in the central shoots. The shoots were then tied up with coconut stipules and kept moist. The seedlings were kept under-observation for a week and the results obtained are given in the following table.

Table I.

| Serial No. | Inoculum                         | No. of seedlings inoculated | No. of seedlings infected | Remarks                                    |
|------------|----------------------------------|-----------------------------|---------------------------|--|
| 1          | <i>H. halodes</i>                | 6                           | 6                         | Very heavy infection.                      |
| 2          | <i>H. halodes</i> plus bacterium | 6                           | 2                         | Slight spotting observed in two seedlings. |
| 3          | Bacterium                        | 6                           | 0                         | —  |
| 4          | Control (Distilled water only)   | 6                           | 0                         | —  |

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Thus it may be seen that the presence of the bacterium in the inoculum gave a measure of protection to the coconut leaves against infection by *H. halodes*.

DISCUSSION

The findings reported in the preceding pages tend to show that in nature there exist several micro-organisms which could produce secretory or excretory products possessing inhibitory properties over other living organisms. Application of this phenomenon known as "microbial antagonism" to plant protection has been attempted with varying degrees of success. Most of these attempts refer to soil-borne diseases. The soil is a dynamic biological complex containing millions of active micro-organisms, some of which are parasitic and detrimental to plant growth, while others are beneficial. During the metabolic processes of these micro-organisms substances are produced which may be destructive to their neighbours. When the net effect of these mutually interacting biological products favours the growth of a parasite, then the latter is able to assert itself and exert its action. If this equilibrium could be disturbed and shifted in favour of an organism antagonistic to the parasite, then, the effect would be to control the parasites (S. A. Waksman 1947). It has been found empirically that many soil-

borne parasites can be suppressed by heavily manuring the infected soils with organic manures as well as fertilisers. A generous but, wise use of fertilisers, is, in fact, one of the best ways of reducing damage caused by many of the fungi attacking plant roots. In Britain, the scab disease of potato is controlled by incorporating large quantities of green manure into the soil (W. A. Millard et al. 1922, 1923, 1926, 1927). In Arizona and Texas in the U. S. A. the cotton root rot disease caused by *Phymatotrichum omnivorum* was successfully controlled by putting in large quantities of hay and other organic matter into deep furrows in the soil well before the cotton is planted (C. J. King, 1937; R. B. Mitchell et al. 1941). In Canada, the strawberry and tobacco root rot and the scab of potatoes were eradicated by ploughing in a crop of soyabeans by doing which, the microbiological condition of the soil appeared to have been modified. (A. A. Hildebrand and P. M. West, 1941). In Australia, where available phosphate limited plant growth, dressings of superphosphate reduced the severity of the "take all" disease of cereals (E. W. Russel loc. cit. p. 217). The basis of all these control measures is a modification of the soil microflora and a stimulation of the mode of microbiological activity of organisms antagonistic to

certain parasites. By putting into the soil, plant and animal residues new types of micro-organisms are introduced which are chiefly saprophytes living on dead matter. The additional energy material supplied in the form of fertilisers encourages the development and antagonistic activity of such organisms. A number of factors are connected with the mechanism of microbial antagonism viz. competition for food, an excess of carbon-dioxide, formation of toxins etc. Since most of the organisms inhabit the soil and since the soil alone is a poor medium the addition to the soil of appropriate nutrient materials is an important factor for their development.

There are also instances of soil plant pathogens being suppressed by inoculating into the soil appropriate cultures of antagonistic organisms, for instance, the success achieved by Russian workers in the "take all" disease of cereals, the work on the control of the cotton wilt in India, of the wilt of melons and the *Sclerotinia* disease of sunflower in France. (Grossbard loc. cit.) The 'damping off' of citrus seedlings was controlled in U. S. A. by inoculating the soil with *Trichoderma* spp.

As reported in this paper an attempt has been made to investigate whether the phenomenon of 'microbial antagonism' could be utilised to control some coconut diseases.

*Helminthosporium halodes* is an important fungus associated with the 'leaf rot' disease of coconuts and our preliminary experiments have shown that the bacterial strain referred to has some protective action against this parasitic fungus. It may be said in this connection that Chadiakov (1935) has shown that bacteria of the genera *Pseudomonas* and *Achromobacter* can control *Fusaria* spp. causing the wilt of flax. Wood has described several bacterial organisms used in the control of diseases of lettuce caused by *Botrytis cinerea* and *Rhizoctonia solani* (R.K.S. Wood loc. cit). Work designed to elaborate the antibiotic principle of the bacterium described by us, and its practical application is now in progress.

#### SUMMARY

(i) An aerobic spore forming bacterial strain probably belonging to the *Bacillus anthracis* group, possessing inhibitory action against fungi associated with the root and leaf disease of coconuts has been isolated and studied.

(ii) The bacterium appears to be able to exert active lytic action on fungal mycelium and probably produces during its metabolic activities some potent fungicidal substance.

(iii) Its presence in a mixed inoculum afforded protection to coconut leaf tissue from infection by the leaf rot organism, *Helminthosporium halodes*.

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(iv) The use of the principle of microbial antagonism in the control of plant diseases is briefly discussed.

(v) Work to further elucidate the above and allied aspects is in progress at this Research Station.

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