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Antibiotics in Agriculture * (Part II)

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Antibiotics in Plant Disease Control: Attempts have been made by several workers to make use of the phenomenon of antibiosis in plant disease control. In 1912 Fawcett found that canker lesions were not produced when walnut branches were simultaneously inoculated with *Pseudomonas Juglandis* and *Dothiorella gregaria*. Hartley in 1921 found that the inoculation of steamed soil with some soil saprophytes resulted in a decrease of the parasitic activity of *Pythium debaryanum* on conifer seedlings. In 1924 Porter found that the incidence of diseases of wheat and flax caused by *Helminthosporium sativum*, and *Fusarium lini* was much reduced by the antagonistic activity of a bacterium. Machacek in 1928 reported that *Penicillium expansum* and a species of *Cladosporium* completely inhibited the growth of *Sclerotinia fructicola* and *Botrytis* sp., respectively. Sanford and Broadfoot, in 1931, reported that the 'take-all' disease of wheat could be controlled by the activities of some soil inhabiting fungi, bacteria and actinomycetes. In the same year Henry found that soil bacteria and fungi could control the foot-rot problem of wheat caused by *H. sativum* and *F. culmorum* in sterilized soil. Weindling, in 1932—'34, demonstrated that *Trichoderma lignorum* markedly reduced the parasitic activity of *Rhizoctonia solani* and *Phytophthora parasitica* on citrus seedlings. Subsequently Carter, Allen and Haenseler, Weindling and Fawcett, Garett, Millard and Taylor and other workers proved beyond doubt the beneficial effects of saprophytic bacteria and fungi on plant pathogens and the possibilities of using them for controlling diseases.

These investigations lead to the direct use of antagonists as well as their indirect use for controlling soil-borne plant diseases. Millard and Taylor, in 1927, could obtain substantial control of potato scab by the application of green manure to heavily infested soil. A series of pot experiments showed that a saprophytic actinomycete, *Actinomyces praecox*, was responsible for the control. Application of soya-bean to infested soil was found to increase the growth of *Trichoderma* which antagonised the scab organism and reduced the percentage of potato scab to a considerable extent. Weidling and his co-workers, during 1932—36, investigated the effect of *Trichoderma* which antagonised the scab organism and reduced the percentage of potato scab to a considerable extent. (Weidling and his co-workers, during 1932—36, investigated the effect of *Trichoderma viride* on *Rhizoctonia solani* and found that the former organism produced an antibiotic, later named gliotoxin, which was inhibitory to the growth of the pathogenic fungus. They demonstrated in a series of experiments that *T. viride* could be inoculated into the soil to control the group of plant diseases caused by *R. solani.* Subsequent work has shown that certain soil amendments favourably influenced the growth of the antagonist and thus indirectly helped in the disease control.)Similarly several soil-borne diseases have been studied in this respect and some limited success reported by various groups of workers in England and U. S. A.

With the isolation and widespread use of antibiotic substances, their use in Agriculture has been taken up by several investigators. During the past 15 years keen interest has developed in this field. This is mainly because of the existing need in agriculture for more effective agents for controlling plant diseases. We do not have any effective remedy for most of the soil-borne fungal diseases except to grow resistant varieties. Because of the nature of their onset and spread, thh control of bacterial diseases offer a great challenge to plant pathologists. Still no remedy is in sight for checking the rapidly spreading virus diseases of plants.

Most chemicals for the control of plant diseases are applied to the aerial parts of the plant and are used primarily against fungal diseases. Their action is usually prophylactic as they form a protective coating over the plant surface and kill or prevent the growth of fungus on the plant. The chemicals in use at present for the above purpose possess low solubility in water and do not enter into the plant sap and are called non-systemic or residual toxicants. In contrast to this type of compounds, some other chemicals exert their action from within the plant, usually at some distance from the point of application. These substances have been called chemotherapeutants or systemic agents. In recent years antibiotics have been tested for use as residual toxicants and as systemic agents and some very encouraging results obtained.

Soil microorganisms are isolated and screened against plant pathogens in cross streak assays. If any promising cultures are isolated, they are studied for antibiotic production in the laboratory under submerged aerated conditions and finally tested in fermentation tanks. The substance in the broth, if produced in sufficient quantities, is isolated into a crude product and tested for its action on the original test organism and also on other plant pathogenic fungi, bacteria and viruses. Then the antibiotic is tested for phytotoxicity on plants. Also, the effect of the substance on seed germination is tested. If found non-toxic it is subject to extensive test in the laboratory and green house. The systemic action of the antibiotic, the time taken for the antibiotic for being absorbed by the tissues, the translocation and stability of the antibiotics in the host tissues, the effect of the antibiotic on other physiological properties of the plant etc., are studied in detail. Then, if found encouraging, the substance is tested in the field for confirming the results obtained in the laboratory and green house.

The possible uses of antibiotics in plant disease control are by way of (1) seed treatment to eradicate the external and internal seed-borne fungi, bacteria and viruses, (2) foliage spray or dust material, to protect the external surfaces of plants from infection as in the case of copper fungicides, (3) soil applicants to eradicate the pathogens in the soil and (4) systemic agents against fungi, bacteria and viruses. Certain amount of succes has been attained in each of these control methods with antibiotic substances, which will be briefly described below:

1. *Seed Treatment:* The aims of treating the seeds with chemicals are three fold (i) to kill the pathogens on the surface of the seed (ii) to kill the pathogens inside the seed and (iii) to protect the seed and seedlings from attack by soil-borne plant pathogens. Several copper and mercurial fungicides are available in the market, which when used for the seed treatment, eradicate the external seed-borne fungi and bacteria and also, to some extent, protect the seed and seedlings from infection by soil-borne organisms when planted in the soil. But these chemicals are ineffective in killing the organisms present inside the seed. The work carried out so far, with antibiotics has shown that they are capable of penetrating deep into the tissues of the seed and selectively inhibit the growth of the pathogen. Cycloheximide has been found to give good control of deep seated fungus *Ascochyta pisi* in pea seeds. Barley seeds were freed from internal infections of covered smut by soaking the seeds with cycloheximide. Helixin B was reported to be effective against internal infections of *H. sativum* in wheat. Streptomycin has been used for seed treatment purposes, particularly for the control of bacterial diseases. Soaking of cucumber seeds infected with the angular leaf

spot bacterium *(Pseudomonas lachrymans)* in streptomycin solution was effective in controlling the disease. Similarly tomato bacterial canker caused by *Corynebacterium michiganensis* was also controlled. The antibiotic also is reported to control internal infections of bean blight *(Xanthomonas phaseoli)* and blackarm disease of cotton caused by *Xanthomonas malvacearum.*

The main draw back in these antibiotics seems to be the toxic effect on the seeds. Most of the antibiotics when used at slightly higher concentrations inhibit the seed germination thus limiting their use for seed treatment.

2. *Foliage spray and dust:* The use of antibiotic for this purpose is relatively less important as a good number of cheap chemicals are already available. However, some antibiotics have been studied for use as protective agents and satisfactory results obtained. Actidione is one of them. This antifungal antibiotic has been found especially effective against powdery mildews and in this respect found superior to Bordeaux mixture and other common spray materials. The antibiotic is also extensively used to control turf diseases where the green colour is desired to be maintained. Bordeaux mixture and other spray materials leave stains on the foliage whereas cycloheximide does not alter the green colour. Similarly streptomycin has been found to protect tomato from bacterial speck. Thiolutin has been found effective against frog-eye disease of apple and late blight of potato.

3. *Soil application:* It is well known to agricultural scientists, for several reasons, that soil-borne plant diseases are the most difficult ones to combat. The organisms may live in the soil as a facultative saprophyte or as a facultative parasite. Any attempt to kill these organisms by the application of chemical agents to the soil may result in drastic changes in the microbial equilibrium thus causing more harm than benefit to the plant growth. There are not many selective agents known which can act only on the plant pathogens when applied to the soil. They are bound to kill other beneficial organisms also. The soil being a complex substratum composing various physical, chemical and biological systems, it is very difficult to successfully apply any chemical substances to the soil for selective activity. Antibiotic substances, being selective agents, have been tested for this purpose, but so far not much encouraging results were obtained. An antibiotic when applied to the soil is inactivated sooner on later for one or more of the following reasons : (i) the substance may be inactivated due to adsorption onto the soil

and clay particles; (ii) it may be inactivated due to the unsuitable pH or other physical properties of the soil; (iii) it may be acted upon by other soil micro-organisms which break them down into substances of no antibiotic value; (iv) the chemicals present in the soil may react with the antibiotic and cause disintegration of the molecule. Of the several antibiotics studied so for, none have been found to be stable enough to act freely on the susceptible microbes present in the soil. Gottlieb and his associates in their studies on the factors involved in the inactivation of antibiotics in the soil, came to the conclusion that they are not produced under natural conditions in the soil. However, recently actidione and gliotoxin were shown to be produced in the soil under certain amended conditions. Wright in her investigations on the production of gliotoxin in the soil found out that *Trichoderma viride* produced, significant concentrations of gliotoxin on the seed coats of legumes buried in the soil. More detailed investigations are required to understand antibiotic production in the soil, especially in and around organic waste particles. Also, the part played by such substances, if at all they are produced, in controlling the plant pathogens and in altering the soil microbial population, is to be evaluated. With our present knowledge we can neither say that it is possible to utilize the phenomenon of antibiosis or antibiotic substances for controlling soil-borne plant pathogens, nor can we reject the idea as an impossible one. It can however be said that at least some of the common soil organisms, like *T. viride*, do play an important role by producing chemical substances on the seed coats, which might result in better germination of the seeds.

4. *Systemic agents :* With our increasing knowledge on the physiology of parasitism of insects, fungi, bacteria, viruses, etc. we are all the time exploring the possibilities of more effectively checking plant diseases and pests. In recent years our attention has been diverted to systemic agents for this purpose. Several chemicals have been tested so far for systemic activity in plants, and a number of them have been selected. Quite a few antibiotics have been found to be systemically translocated in plants. It is this property of the antibiotics that is most important for a plant pathologist who is looking for a chemical to combat fungal, bacterial or virus diseases which are soil-borne or systemic and difficult to combat. Good number of investigations have been carried out in U. S. A., U. S. S. R., U. K. and other places and very encouraging results obtained. Though we have not yet got the ideal antibiotic substance for controlling all the difficult diseases of plants, a few of the substances have been found quite effective in checking important diseases.

Before we go into the details of these substances it is necessary that we should know something about the ways and means of studying the systemic activity of the antibiotic substances, their rate of translocation, stability inside the tissues, phyto-toxicity, etc.

Systemic activity: Several techniques are available to study the systemic translocation of antibiotic substances in plant. The important ones are (i) guttation technique (ii) leaf-disc assay method and (iii) plant-sap assay method.

(i) *Guttation technique:* The plants are fed with aqueous solutions of antibiotics through the root system and, after periodical intervals, guttations are collected in the morning hours. The guttation from the control plants is taken as check and the prevalence of antibiotics in the water examined by the spore germination method. The guttations are placed in grooved slides and spores of the test organism allowed to germinate in them. The percentage and rate of germination are studied. In the studies with bulbiformin the characteristic bulb formation in the germ tubes due to the antibiotic was used to identify the presence of the substance in the guttations of treated plants. The limitations of this technique are that (a) not all plants produce guttation easily, (b) the guttations are to be collected only in the early hours of the day and (c) that in some cases the antibiotics inhibit the formation of guttation itself. Bulbiformin at high concentrations was found to prevent the exudation in tomato plants.

(ii) *Leaf-disc assay method:* In this method, firstly the plants are sprayed or dipped in the antibiotic solutions. After periodical intervals representative samples of the leaf, stem or other parts of the plants are selected and washed thoroughly in running water. Then they are assayed in agar plates impregnated with test organisms. The antibiotics, if present in the tissue, will diffuse into the agar medium and inhibit the growth of the test organism, around the leaf tissues. This is a very useful and easy technique, but the limitation for the technique is that in some cases even the normal plant tissues exert an inhibitory effect on the test organism. Even then, if the inhibition zone is relatively more around in the treated tissues as against the check, it can be taken as an indication of the presence of the antibiotic in the tissues. As an alternative different test organisms can be studied and one which is not sensitive to the normal plant tissue is selected for the study.

(iii) *Leaf-sap assay:* The plants are treated as detailed above, washed thoroughly and the sap extracted, with or without addition of small quantities of water. The sap is then assayed for the antibiotic by dipping in a paper disc and plating on agar plates impregnated with test organisms, or by pouring into stainless steel cups placed on such agar media. The antibiotic, if present in the sap, would diffuse into the agar medium and cause inhibition of growth leaving a clear ring around the paper disc or the steel cup, as the case may be. The main limitation of this test is that we need a fairly good amount of plant material to obtain the required quantity of sap, thus making it difficult to study the leaf-wise translocation of the antibiotic in a plant.

Phytotoxicity: The antibiotic may by highly useful against fungi, bacteria, viruses etc., and may be systemically translocated in the plants, but it will be of no value to the plant pathologist, if it is toxic to the plants at minimum inhibitory concentrations for the pathogens. It is therefore important that the antibiotics are thoroughly investigated for phytotoxicity before taken up for use in plant disease control. They can be tested by spraying various concentrations or by dipping the in-tact-leaf tissues into aqueous solutions and observing the changes in the plant. The usual toxic symptoms observed are that chlorotic spots develop on the leaf-blade and on other plant parts and also in some cases the tissues are malformed. In some cases it has been reported that this toxicity could be overcome, to some extent, by spraying graded doses of the substance at regular intervals, thus training the plant to withstand the toxicity. This has been observed in the case of streptomycin sulphate on acid lime plants. If the plants are sprayed successively with 250 ppm. 500 ppm. and 1000 ppm. of the antibiotic at an interval of 8 to 10 days, there is relatively low toxic symptom than when sprayed directly with 1000 ppm. of the antibiotic. Also, if the plants are sprayed repeatedly with 1000 ppm. of the antibiotic, the intensity of the chlorotic symptoms fades away slowly and after the fourth or fifth spraying there is practically no toxic symptom on the leaves, the original chlorotic spots disappearing after two to three weeks.

Field Test: When once the antibiotic is found satisfactory in the laboratory and green house studies, it is put for more intensive investigations in the field. It may be that the substance is not stable to heat and light and thus disintegrates when sprayed on the plants in the field. Penicillin is known to be thermolabile and so is not very useful for spraying in the field. Filipin, which was reported to be highly effective under laboratory and green house conditions, was found ineffective in the field, which was mainly because of its instability in the sun light. It is also to be understood that the conditions of test in one country are not always the same as existing in another and in larger countries, from one region to another, so all attempts should be made to study them in detail and if necessary isolate new antibiotics for use under given set of conditions.

Present Position of Antibiotics in Plant Disease Control: Of the good number of antibiotics tested so far streptomycin and other agricultural preparations containing the antibiotic, viz. Phytomycin, Agrimycin, Agristrep etc. are the most outstanding ones which have come into wide use in U. S. A. for controlling bacterial diseases of plants. They are used for eradicating seed-borne bacterial infections of fruit trees and vegetable crops. Fire blight of pear and apple is one of the serious bacterial diseases caused by *Erwinia amylovora*. It is mainly transmitted from one tree to another by honey bees which visit the flowers. By placing a small trap containing streptomycin dust at the mouth of the bee-hives, and making the bees pass through the trap, the spread of the disease in the orchards is effectively checked. Of the other antibiotics which have come into use are actidione, an antifungal antibiotic against several fungal diseases, gliotoxin, an antibacterial-cum-antifungal substance against some diseases of fruit trees, and some unnamed substances reported to be used by the Russian Workers for controlling root-rot fungi. The latter antibiotic, when sprayed on the foliage, is said to be translocated mainly to the root region and thus prevent infections by the soil-borne root-rot fungi. Several other substances like mycothricin, helixin, streptothricin, candicidin, thiolution, etc. have been reported to be useful for the plaut disease control. But more detailed investigations are required before they are put into wide use, the main limiting factor being large scale production of the antibiotics at cheap costs for use in agriculture.

Another important aspect of the use of antibiotics in plant disease control is the inhibitory effect of a few of these substances on plant viruses. Streptothricin, terramycin and bulbiformin have been found to be quite effective in checking plant virus infections. When the virus sap was mixed with the antibiotics and inoculated on the respective host, it failed to infect the plants thereby indicating the inactivating power of the chemicals. The conditions leading to the inactivation of the viruses by the antibiotics are to be studied in detail before they are applied for controlling the diseases. Considering that no chemicals are available for effectively checking virus diseases of plants, antibiotics, especially because of their systemic translocation in the plants, appear to be most promising agents for the purpose.

Antibiotics as Plant Growth Promoters: Besides their usefulness for plant disease control, some of them are known to promote growth in plants. Penicillin is known to cause such an effect in tobacco and other plants. Gibberellic acid, which was originally isolated as an antibiotic substance from *Gibberella fujikuroi*, has been found to possess so much of growth promoting effect on many plant species, and such a great interest has been developed in its use as plant growth regulator, that now, it is known more as a plant hormone than as an antibiotic.

Some workers have also studied the production of toxic substances by plant pathogenic organisms *in vivo*, which substances, incidentally happen to possess some antibiotic properties. Because of their nature and physiology of production, these substances are to be designated as plant toxins rather than antibiotic substances. In a recent report Tveit stated that when he inoculated several pathogenic and non-pathogenic species of *Chaetomium* on oat seedlings, he found that some of the non-pathogenic ones produced the antibiotic chetomin *in vivo*. This antibiotic is mainly an antifungal agent which prevented infections of the seedlings by *Helminthosporium victoriae*, *H. avenae*, etc. If such phenomenon are made use of, it would be quite possible to control many of the troublesome plant diseases.

Use in Allied Fields: (1) *Food Preservatives:* It has been reported that some of the antibiotic substances could be used in food preservation and also in the canning industry with great advantage. Streptomycin at 0·1 percent strength could preserve spinach and cole slaw in the frigidaire for a longer duration. Addition of 0·1 per cent aureomycin to the ice blocks helps increasing the keeping quality of fish; the antibiotic present in the ice has been found to arrest the growth of bacteria which usually attack the fish. Injection of aureomycin into beef-animal few days before they are taken to the slaughter house is found to increase the keeping quality of beef.

In the canning industry spore forming bacteria play a devastating role. The food stuff is to be heated to 80°C or above to kill the bacteria contaminating it, but this heat treatment affects the quality and flavour of the food stuff. Addition of subtilin is known to substitute the heat treatment and thus helps in preserving the quality and flavour of the product.

(2) *Fermentation Industry:* Antibiotics have been successfully used in fermentation industries to overcome microbial contaminants. Polymyxin has been found to exert selective action

on gram-positive and gram-negative contaminants of beer industry. In whisky industry the malt is commonly found to be contaminated by bacteria causing sliminess of the mash. This is overcome by adding penicillin or a mixture of penicillin and polymyxin to the medium. Similarly fungal contaminants can be put down by the use of antifungal antibiotics.

(3) *Animal Growth Promoters:* In an attempt to sterilize the intestinal tract of chicks with streptomycin, Moore and his associates discovered in 1946 that the antibiotic actually increased the growth of the birds. Subsequent work by other investigators brought out that penicillin, aureomycin, terramycin, etc. could also induce better growth, upto 15 per cent in the birds. This growth promoting effect was more in the young birds than in the older ones. Crude wastes in penicillin factories which contain small quantities of pencillin is being extensively used for feeding the chickens. It has also been found that some antibiotics could induce better growth, upto 20 per cent, in turkey, swine, young calves, etc., when added on to the feed. This means the birds and animals go to the market sooner than usual and thus help saving labour and cost of production.

Several explanations have been given for this inducement of growth. It is believed that the antibiotics, besides removing the microbial competition with the animal body for food material, also promotes synthesis of essential substances like B-complex vitamin in the tract.

(4) *In Beneficial Insects:* The growth promoting effect is not confined to the birds and animals. It has also been shown in some insects. Honey bees fed with fumigatin are reported to produce 15 to 25 per cent more honey. Likewise aureomycin-fed silk worms produce 10 to 14 per cent more silk than the normal ones.

Besides the above uses for antibiotics, several important utilities have been invented recently. They are successfully used in paints, floor wax, paper mills etc., to cut down the development of moulds and bacteria. In the biological studies in the laboratory it is used to selectively inhibit the growth of unwanted organisms. For isolating bacteria from the soil or water antifungal antibiotics are mixed with the media and for isolating the fungi antibacterial antibiotics are used. It is also used to check the growth of yeasts in sugar media fed to lac insects. Thus it could be seen that the field of antibiotic, though only less than two decades old, has spread its roots into various branches of science, and is at present engaging the attentions of several scientists throughout the world.