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Antibiotics in Agriculture * (Part I)

by

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Introduction: The earliest knowledge of the antogonistic properties of microbes can be traced to ancient times. Folklores, stories abound among several peoples, to the effect that cheese, mouldy bread and other spoiled food products can be used for controlling human and animal infections. But specific references to antagonistic effect of one microorganism on other could be found in the literature only during the later half of last century. Vuillemin appears to have been the first to use the word 'antibiosis' in the year 1889 to describe the phenomenon of 'the survival of the fittest' among microbes. Marshall Ward in 1899 adopted the word to describe microbial antagonism. Since then people have been using the words 'antibiotic substance' 'toxic substance', 'lethal principle', 'inhibitory principle', 'staling product', 'bacteriostatic substance', 'antimicrobial substance', etc, to denote the chemical action, now known to be due to antibiotics. The word 'antibiotic' was used as an adjective in the early years to designate 'processes which are against life'. Starting from 1941 Waksman and his collaborators used the word 'antibiotic' to denote the chemical substance. In the year 1945 Waksman cleared the confusion that existed in the definition of the word 'antibiotic' by enunciating a clear cut definition. According to him '*an antibiotic is a chemical substance which is produced by a microorganism and which has the capacity, in dilute solutions, to inhibit the growth of and even to destroy other microorganisms*'. Since then this definition has been well accepted by scientists throughout the world and the word antibiotic has firmly established even in the lay minds. In recent time this word has been used to include chemical substances produced by plant and animal tissues, but such expansions of the definition are to be discounted as they will lead again to confusion. The chemicals from plants which possess antagonistic effect on microbes can be called 'plant antibiotics' or 'antibiotic-like sustances' as suggested by Waksman.

During the past two decades antibiotics have revolutionized the medical science. Several deadly diseases are now successfully cured by administering these substances. The achievements with antibiotic

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substances have been so remarkable that, medically speaking, the present age is called 'antibiotic age' or 'antibiotic era' and the antibiotic sustance the 'miracle drug' or 'wonder drug'. With the establishment of antibiotics in medicine, their use in other fields have been explored with all vigour. The results obtained so far indicate that antibiotics have come to occupy a prominent place in animal nutrition, agriculture and in many of the biological and chemial sciences.

Historical Review: Though there is a tendency to attribute the discovery of antibiotic substances to certain individuals, the historical background goes to show that our present knowledge of antibiotics has accumulated from a complex origin. The efforts of scientists working on the microbiology of man and animal systems, the soil, water and sanitation and plant diseases laid the foundation for the science. The advances in chemotherapy brought to light the selective activity of chemical substances on microorganisms; salvarsan and sulfa drugs were successfully used for eradicating the parasite without affecting the host. The progress made in the chemistry of isolation of natural and fermentation products also contributed to the development of isolation procedures for antibiotic substances. So, unlike in other fields, no one person can be called 'Father of antibiotics'.

As early as in 1871 Lister used bacterial cultures for treating wounds. Roberts in 1874 observed that liquids in which *Penicillium glaucum* was growing luxuriently could with difficulty be artificially inoculated with bacteria. In 1876 Tyndall came out with the 'struggle for existence' theory among organisms growing in a mixed population. He found that bacteria which produced green pigments were highly inhibitory to *Penicillium*. The following year, in 1877, Pasteur and Joubert made very important observations; they found that artificial reproduction of anthrax in susceptible animals could be repressed by the simultaneous inoculation with various saprophytic bacteria. On the basis of these observations Pasteur suggested: "One can infect abundantly an animal with anthrax without the animal becoming diseased; it is sufficient that the fluid contains in suspension simultaneously the anthrax organism and a common or harmless bacterium. These facts perhaps justify the highest hopes for therapeutics". In 1879, de Bary, who is considered to be father of Plant Pathology, emphasized the significance of mutualistic and antatgonistic relationships among fungi occurring in nature.

Since these early observations, several workers have attempted to explore the possibilities of utilizing the phenomenon of antibiosis for the benefit of mankind. Cantani, in 1885, found that patients suffering from tuberculosis benefited from treatment with a saprophyte, *Bacterium thermo.* Babes' in the same year observed that certain microbes could produce chemical substances both in liquid and solid media, which had the capacity to inhibit other organisms and he suggested that a study of the chemical might lead to treating one bacterium with another. Garre in 1887, and later other workers, isolated antagonistic pyocyaneous organisms and used them to check infections by pathogens. In 1889, Charrin and Guiguard demonstrated that *Pseudomonas pyocyanea* produced a soluble product that destroyed anthrax bacillus but did not affect red blood cells, thus bringing out the selectively inhibitory property of the chemical substance. In 1899 Emerich and Low for the first time isolated a chemical substance, named by them as pyocyanase, from an old culture of *P. pyocyanea,* which had a marked inhibitory effect on numerous bacteria, but had no injurious effect upon animals when injected intravenously. But they believed that the chemical was an enzyme and not a distinct antibiotic substance as known at present. The interest in pyocyanase grew rapidly but several attempts made to put it into practical utility failed because of its toxicity. In the meantime, in 1907, Nicolle established that filtrates of *Bacillus subtilis* cultures had lytic effect on various pathogenic bacteria including pneumococci. Rappin, in 1912, reported that *B. mesentericus* types of bacteria were active against tuberculosis organism. Much, in 1925, obtained a substance from *B. mycoides* which was used with limited success for treating various infectious diseases.

As early as in 1896 Gosio recorded the isolation of a crystalline compound from cultures of *Penicillium brevicompactum* type which inhibited the growth of *B. anthracis.* In 1897, Duchesne demonstrated *in vitro* and *in vivo* the beneficial effects of inoculating *P. glaucum* against pathogenic bacteria. But these two important observations on the antagonistic property of fungi were not pursued any further at that time.

In 1890, Gasperini for the first time reported on the inhibitory effect of actinomycetes on bacteria. Greig-Smith, in 1917, observed that certain actinomycetes inhibited both bacteria and fungi. Lieske, in 1921, reported on certain actinomycetes antagonistic to bacteria, and pathogenic to man. Thus it can be seen that prior to the report

of Fleming on penicillin in 1929 there were several observations and serious attempts to make use of the phenomenon of antibiosis. In 1929, Fleming published his 'chance' observations on the effect of a fungal contaminant, later on identified as *Penicillium notatum*, upon the growth of bacteria in an agar plate. The fungus brought about lysis of neighbouring staphylococcal colonies. The culture broth had the capacity to kill or inhibit the growth of a number of human pathogens. Fleming named the antibacterial agent as pencillin and also suggested that the substance may be valuable in treating bacterial infections. This import work was burried in the literature for nearly 10 years. During this ten year period, however, attempts were made by Clutterbuck and his associates and Reid to isolate the chemical substance but they failed mainly because of the thermolabile nature of the substance.

In 1939, Dubos, one of the early students of Waksman working at the Rockefeller Institute in New York, isolated from *Bacillus brevis* the antibacterial polypeptide, tyrothricin complex, and clearly demonstrated the antibiotic properties of the substance and its possible use. Simultaneously, the Oxford team of workers headed by Chain and Florey, who were searching for a chemical substance active against *Staphylococcus* and *Streptococcus* infections decided to investigate penicillin and after a great struggle succeeded in their attempts to isolate the substance. Considering the meager quantity of the antibiotic produced by the strain in liquid broth, and its thermolabile nature, it is to be appreciated that the investigators did commendable work in establishing the usefulness of the substance in medical science. Subsequently, in 1940—'41, in a systematic study undertaken at the Northern Regional Research Laboratory in U. S. A., strains of *Penicillium chrysogenum*, which are better yielders of penicillin, were obtained. It was in that laboratory the submerged culture technique and other improvements in the methods of production and isolation processes, were developed. Due to the painstaking work of several investigators high yielding strains of *P. chrysogenum* were obtained and at present the strains used in the industry are capable of producing over 4000 units/ml as against about 100 units/ml produed by the strain isolated by Fleming. All these improvements lead to its wide spread use in medicine and subsequently in other fields.

Though Waksman and Foster recorded in 1937 their observations on antagonistic action of some actinomycetes against fungi and bacteria, it was only in 1939 that Waksman and his

students launched an organized search for actinomycetes active against gram-negative and acid-fast bacteria. They found, early in their studies, that soil actinomycetes formed a great potential source for antibiotic production as 20 to 30 per cent of the isolates examined by them were found to inhibit bacteria or fungi or both. Between the years 1939 to 1943 many soil actinomycetes were screened by this group of workers and several antibiotics including actinomycin, clavacin, fumagacin, micromonosporin, and streptothricin, were isolated but all of them proved too toxic for use in the animal body. However they continued their work with all enthusiasm and in January 1944 announced, the isolation of streptomycin with activity against gram-positive, gram-negative and acid-fast bacteria, including *Mycobacterium tuberculosis*, and with relatively low toxicity to animal. In the early stages of its development as a chemotherapeutic agent, the workers on streptomycin had to face many odds but very soon its importance was realized and a one-million dollar programme was launched by the industrial concerns to establish it in the medical field. At present streptomycin, together with para amino salycilic acid and isoniazid, forms the formidable weapon to fight tuberculosis throughout the world. Streptomycin and dihydrostreptomycin (a catalytic hydrogenation product of streptomycin) are generally administered for treatment against several gram-positive and acid-fast organisms.

The great success attained by streptomycin gave incentive for an extensive screening programme by industrial concerns to isolate soil actinomycetes with antagonistic properties. Thousands of soil samples have so far been tested and millions of actinomycetes screened for activity against other microbes. In all, nearly 150 antibiotics have been reported from this group, of which the important ones are chloramphenicol, aureomycin, terramycin, tetracycline, neomycin, erythromycin, carbomycin, azoserine, sarkomycin, thiolutin, nystatin and candicidin. Besides these, bacteria and fungi have yielded over 200 antibiotic substances. The potentialities of many of these substances have not been thoroughly investigated. Actinomycin which was isolated in 1940 by Waksman and Woodruff and rejected by them because of its high toxicity, has now been found useful against neoplastic tissues like lymphatic system. It is believed that with more and more work newer and better antibiotics will be and wider use for them isolated discovered.

Isolation of Antibiotics: Among the microorganisms, the most important antibiotic producing groups are bacteria, actinomycetes and fungi. They are all distributed in nature, in the soil, air and

water, but soil forms the main source for most of these organisms. Except a few fungi and bacteria, all other antibiotic producing microorganisms have originated from the soil. Among the bacteria, members of the genus *Bacillus*, and *Pseudomonas*, spore formers and non-spore formers, respectively, are extensive producers of antibiotics: nearly 25 antibiotic preparations have been isolated so far from strains of *B. subtilis* alone. Among the fungi, species of *Penicillium* and *Aspergillus* are particularly important as several of their strains produce penicillin. Among the actinomycetes, species of *Streptomyces* are of great importance because of their potentialities to produce antibiotic substances. So far they have yielded over a dozen antibiotics of high pharmaceutical value.

Though penicillin was discovered by the 'chance' observation of Fleming, subsequent workers have evolved, by constant research, several new techniques for secreening microorganisms for antibiotic production. With the help of these techniques it has been made possible to test millions of isolates of microorganisms for antibiotic properties. When once an organism has been selected for the production of an antibiotic the subsequent steps, comprise of the development of suitable media, study of the conditions favourable for the growth and production of the antibiotic in the laboratory and subsequently in the fermentation tanks, improvements in the method of isolation and purification processes, etc. The antibiotic thus obtained is then tested for its biological, chemical and physical properties, and if found encouraging, put for animal test. It is not within the purview of this paper to go into the details of various animal tests that are to be conducted before an antibiotic is tested for therapeutic purposes.

Chemical and Physical properties of Antibiotics: Antibiotics vary greatly in their chemical composition. Some of them are simple substances containing C, H and O, such as clavacin ($C_7H_6O_4$) and kojic acid ($C_6H_6O_4$). Others are more complex and contain nitrogen as in streptomycin ($C_{21}H_{37}O_{12}N_7$) or nitrogen and sulfur, as in penicillin ($C_9H_{11}O_4SN_2R$), or nigtrogen and clorine as in chloramphenicol ($C_{11}H_{12}O_5N_2Cl_2$). Because of their great complexity, the chemistry of most antibiotics offers a real challenge to the students of organic chemistry. On the basis of what we know about their chemistry we can broadly classify antibiotics into following groups:

1. Amphoteric substances, eg., the penicillins.
2. Basic substances, eg., streptomycin, neomycin.
3. Polypeptides, eg., polymyxin, gramicidin.

4. Quinones, eg., rhodomycin, xanthomycin.
5. Polyenes, eg., candidin, nystatin.
6. Tetracyclines, eg., aureomycin, terramycin.
7. Phenolic substances, eg., griseofulvin, gliotoxin.

Some of the important antibiotics have been studied in detail and their molecular structure elucidated. Of these only three antibiotics have so far been synthesized, namely, penicillin, clavacin, and chloramphenicol. Most of the antibiotics are single chemical entities, while others are made up of several closely related compounds, eg., geomycin complex, mycothricin complex, polymyxin complex.

The physical properties of these antibiotics also vary to a great extent; while some are colourless others are coloured with absorption maxima between 290 to 400 hh. The polypeptide antibiotics are highly stable while the penicillins are less resistant to heat. The stability of antibiotics at various hydrogen-ion concentrations in aqueous solutions vary to a great extent. Most of the antibiotics are stable in sunlight but a few like filipin disintegrate quickly in the light. Melting point of antibiotics also vary to a great extent and forms a basis for identifying some of the better known substances.

Antimicrobial Activity: The antimicrobial property of an antibiotic is of the greatest importance to us. Some antibiotics are characterized by a very narrow antibiotic spectra and others possess very wide spectra. Viomycin is active mainly upon mycobacteria and do not have much effect on other microbes, whereas gliotoxin and clavacin are active upon a large number of bacteria and fungi. Most of the other antibiotics fall between these two extremes. Penicillin is active mainly against gram-positive bacteria, streptomycin and related antibiotics upon gram-negative and gram-positive bacteria, including mycobaceria and actinomycetes. Chloramphenicol, aureomycin and terramycin are active upon rickettsial and some larger viruses, besides many bacteria, and are frequently spoken of as 'broad spectrum antibiotics'. Actidione, candicidin, etc., are active mainly upon fungi but not upon bacteria. Also, within each group of sensitive organisms there is a wide range of specificity or sensitivity to a given antibiotic. The antibiotic spectrum, therefore, forms an important basis for characterizing an antibiotic substance. Though an ideal antibiotic would be one which possess a broad spectrum against bacteria, mycobacteria, fungi, viruses etc. with low toxicity

in animal and other favourable pharmaceutical properties; from what we know now of antibiotics, the chances of isolating such a substance seem to be very remote.

Mode of Action : Most antibiotics at low concentrations exert an inhibitory effect on the sensitive organisms, and at higher concentrations a cidal or lethal effect, depending upon the duration of contact of the chemical with the organism. Streptomycin is bacteriostatic to many bacteria at low cencentrations but kills at least some of them at high concentrations. Candicidin is fungicidal to *Candida albicans* whereas griseofulvin is mainly a fungistatic agent, being incapable of killing fungi even at higher concentrations. These variations may be explained by the mode of action of the antibiotics on the cells of micro-organisms.

The nature of the agent, the stage of growth of the bacterial cell, the composition of medium or substrata, the environmental factors, all influence greately the mode of action of antibiotic on a given organism. Various theories have been proposed to explain the mechanism of action of antibiotics on microbes, but the important ones are: (1) the antibiotic enters into a competitive inhibition, substituting for one of the essential nutrients, (2) the antibiotic interferes with the assimilation mechanisms such as protein synthesis or glucose metabolism, thereby upsetting the energy mechanism of the microbe, (3) the antibiotic interferes with the enzyme system affecting notably respiratory mechanism of the bacterial cell and (4) the antibiotic may interfere with the cytoplasmic membrane of the cell. But it is bilieved by most workers that the antibiotics mainly interfere with the enzyme system. Chloromycetin, tetracyclines, streptomycin and penicillin are some of the important antibiotics so far studied for their mode of action. Chloromycetin is known to interfere with protein synthesis and utilization of fats and esters but even at high concentrations the formation of nucleic acids, polysaccharides and many processes of dissimilation are not affected. In the case of tetracyclines, it is believed that the chemicals interfere with protein synthesis and nucleic acid metabolism. The work so far carried out on streptomycin has revealed that the antibiotic interferes with nucleic acid metabolism and also with the Krebs cycle. In the case of penicillin the interference is apparently concentrated in the nitrogen metabilism of cells. Penicillin is more effective on gram-positive bacteria than on gramnegative bacteria. Gram-positive bacteria are more exacting in their nutritional requirements than the latter group. Penicillin is known to interfere with

the uptake of glutamic acid by gram-positive bacteria, for which glutamic acid is essential for protein synthesis, whereas gram-negative bacteria, even in the absence of glutamic acid, can build up protein. It is also reported that penicillin is absorbed by the sensitive bacterial cell and bound with the cytoplasm which at higher concentrations killed the cell.

Development of Resistance to Antibiotics: It is known for some years that certain strains of sensitive organisms, when allowed to grow unmultiplied in the presence of high concentrations of a drug, are capable of developing resistance to the drug. This problem of bacterial resistance to chemotherapeutic agents received special attention with the increasing utilization in recent years of penicillin and streptomycin. It also gave a fillip to detailed investigations into the fundamental aspects of drug resistance. It has now been established that the phenomenon of drug resistance is a genetic character. Several new techniques were developed and important experiments were carried out during 1949 — '55. The results have thrown valuable light on the development of resistance to antibiotics.

The occurrence of resistant cells in nature has been brought out, thus establishing that antibiotics acted mainly as screening agents to detect the naturally occuring resistant cells and that they do not act as mutagenic agents. In nature the resistant cells are believed to occur in the ratio of about 10-7 to the sensitive cells. Also two distinct patterns of resistance viz., the penicillin pattern and the streptomycin pattern have been recognized. In the former case there is a stepwise increase in the development of resistance with increase in antibiotic concentration while in the latter type the resistance may not always follow any stepwise pattern, the intermediary stages being absent in the development processes.

Several theories have been formulated for the development of resistance to antibiotics. The importont ones are: (1) There is a certain degre of spontaneous mutation taking place in nature due to irradiation in the sunlight. The mutation rate in bacteria is believed to be high because of the large molecules of DNA present in the cell. (2) The antibiotic may act as a mutagenic agent, affecting the nucleus or nuclear material (3) the antibiotic may bring about a change in the cytoplasm leading to adaptation of the cell to the changed environment. Experimental evidence obtained so far indicates that there is some truth in each of the above theories, but more work is

required to understand these complicated genetic changes in bacterial cells. It has also been observed that in some cases the susceptible cell produces an adaptive enzyme to utilize the antibiotic for its nutrition. Penicillinase is produced by *Staphylococcus aureus* which enables the organism to utilize the substance. Similarly new mutants arise from susceptible species of bacteria, which are not only resistant to the antibiotic but become dependent mainly on the chemical for its growth. Streptomycin-dependent-strains of *Escherichia coli* have been isolated and used for several fundamental studies. Similar phenomenon has also been observed in the genetics of development of resistance to insecticides.

Antibiosis in Natural Processes : Natural processes due to biological reactions are largely a result of growth and activities of animals, plants and microbes inhabiting this planet. All forms of life, under certain natural conditions, produce chemical substances which are either benefical or harmful to other living beings. Thus there exists an interdependent or antagonistic relationships among various groups of living beings. But in general a system of biological equilibrium is maintained in nature, lest there be undue multiplications of one or other sets of organisms. The life span of microorganisms is relatively shorter than that of other living groups and so there exists a competition among the various microorganisms for their survival in nature, especially in the soil. Several soil microbes are now known to produce antibiotic substances in the laboratory under given sets of conditions, which are much different from those existing in the soil. Therefore, it is not clear whether antibiotics play a great role in the 'survival of the fittest' theory among soil microbes. According to Karassilnikov antibacterial substances form one of the most potent weapons in the struggle for existence among microbes. But according to Waksman these assumptions are mainly speculative on the basis of the existing knowledge. The nutritional requirements of the antibiotic producing organisms do not exist in the soil and even if traces of antibiotics are produced they are likely to be disintegrated immediately due to the complex physical, chemical and biological factors acting on them. If the reverse is true only certain type of bacteria or actinomycetes could have been expected to be predominant in the soil, and the remaining ones would have developed resistance to the antibiotics.

Recent investigations, however, have clearly indicated production of antibiotic snbstances by microorganisms under natural conditions.

Tveit in 1956 found that the simultaneous or prior growth of the saprophyte *Chaetomium globosum* with *Puccinia graminis* the pathogen causing rust disease in wheat, will suppress the development of the disease on the host plant. According to Brian and his co-workers *Penicillium expansum* produced the antibiotic clavacin in apple fruits under normal conditions. Wright has shown that high concentrations of gliotoxin are produced by *Trichoderma viride* in and around particles of organic matter, such as pieces of straw and seed coats burried in the soil. Good number of antibiotic-producing organisms, including *Penicillium notatum* and *P. chrysogenum* among fungi, *Bacillus subtilis, B. brevis* and *B. polymyxa* among the aerobic spore formers, *Streptomyces griseus, S. lavendulae* and *S. fradiae* among actinomycets, are found in abundance in the ordinary garden and field soils. One could argue, therefore, that such antibiotic formers, growing on plant and animal residues in the soil may produce antibiotic substances at least in a limited scale. Of what importance these antibiotics are in natural processes in maintaining the balance of microbial population ? More elaborate studies are required before any answer could be given to this question. However, it is to be understood that antibiotic production by certain micro-organisms under controlled conditions in the soil could be induced. How far this possibility can be utilized for the benefit of plant growth and for the benefit of mankind can be estimated only with more explorations in the field.