

## Fungicides and their interactions with non-target organisms\*

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I feel greatly honoured for asking me to deliver the Professor S. R. Bose Memorial Lecture for this year, though I feel diffident about my eligibility. Firstly because I have personally contributed nothing to the field in which Professor S. R. Bose has kept himself engaged for nearly half a century from 1918. Secondly because my personal contact with him was just for one year, 1952-1953.

I can still vividly recollect my first meeting with Professor Bose. Immediately after our M.Sc. results were published, our reverend teacher Dr. S. N. Banerjee told me and my batchmate Asoke Das Gupta to meet Professor Bose in his R. G. Kar Medical College laboratory evidently for an employment possibility. So, one day with trembling hearts we went there and met him. To our surprise, both of us were asked to join his laboratory immediately. In March 1952, I joined as a Junior Research Assistant in a CSIR scheme on Antibiotics from Higher Fungi, and Asoke joined as the Kirtikar Research Fellow. We felt the hands of Dr. Banerjee working behind the screen which provided us the opportunity of associating ourselves with the Professor. As colleagues, we had Mr. M. S. Balakrishnan who was a student of M.O.P. Iyenger and Dr. Anil Choudhuri, an elderly medical man who used to spend about three hours every day performing the antibiotic assays. Another gentleman, named Mr. Projesh Sen was an occasional visitor to the laboratory and used to give us tips regarding how to handle Professor Bose. He was a practicing lawyer of the Small Cause Court.

Professor S. R. Bose was truly a legend in his life time. Immaculately dressed up from top to bottom, with a rimless pince-nez on the bridge of his nose and a full-sized Burma cigar stuck between the teeth he used to present an awe-inspiring figure when he walked back home in late hours through the R. G. Kar Medical College campus. As it is well-known, he spent the entire period of his active life as the Professor of Botany at this college where only I.Sc. course was taught. Most of his time was spent in research. During my stay in his laboratory, I never found him absent, except for about a week when a tragic event in his family kept him away from his work. But he came over the shock bravely and reappeared with his usual external calmness. This surprised us, because he was well over sixty at that time.

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\*Professor S. R. Bose Memorial Lecture 1995 delivered on 22 November, 1995

Professor Bose possessed a wide spectrum of interest in the plant sciences, though his major area was Mycology and specifically, the Basidiomycetes. His contributions to this fungal group have become classical and need no mention to this audience. Inspired by the discovery of penicillin, he turned his attention to the antibiotics in the middle of 1940's, when antibiotic research was still at infancy. He initiated research on antibiotics from the higher fungi in India. As you know, very few fungi have proved to be good source of clinical antibiotics. So, Polyporin reported from *Polystictus sanguineus* in 1944 and Campestrin from *Psalliota campestris* failed to stand the test of time. Similar was the fate of many other antibiotics reported by other workers. In retrospect, perhaps we can say that Professor Bose did not select the appropriate organisms for isolation of potent antibiotics. But, at the same time we must appreciate his foresight and awareness to the fact that fungi could be exploited for delivering goods and services to the mankind. Incidentally, this idea forms the basis of modern biotechnology. I pay my homage and respect to this great scientist who initiated me into research.

The realization that useful plants which provide food, fibre, fuel and medicine for man and feed for domestic animals need protection against natural biological destroyers like insects, fungi, bacteria, viruses, nematodes and undesirable weeds dawned upon man ever since such plants were cultivated. To-day, the use of chemical agents, called pesticides, has become an integral part of modern agriculture. In spite of the use of pesticides, the losses caused by various pests to crops are still enormous. According to WHO estimates such losses range from 10 to 30% in North America, Europe and Japan, but in developing countries of Latin America, Africa and Asia the losses are substantially more, e.g. rice 28 to 57%, wheat 30 to 42%, potato 44 to 62%, sugarcane 44 to 71% (Edwards, 1986). Pesticide use and crop yield have been found to have a positive correlation. Japan uses the maximum quantity of pesticides and has also the highest productivity (5.5 T/ha), followed by USA. It is no wonder, therefore, that in order to cope with the increase in world population, the production of pesticides has steadily increased. In 1945 the world production was  $81.8 \times 10^3$  mt and in 1980 it was  $450 \times 10^3$  mt. Despite the early warnings that over-reliance on chemical control might produce undesirable side effects and despite the concern expressed by the environmentalists, the world production of pesticides has increased unabated not only quantitatively, but also in their numbers.

The fungicides constitute a class of pesticides and their target should be the plant pathogenic fungi. A fungicide has been defined by Dimond (1952) as a chemical substance which has the ability to check the damages caused by fungi to growing plants and their products. The first fungicide to be commercially marketed was Bordeaux mixture (Millardet, 1882). About half a century later, the first organic fungicide (dithiocarbamates) was patented by Tisdale and Williams (1934). After another 30 years, systemic fungicides in the form of aminopyrimidines, carboxyimides and benzimidazoles appeared in 1966. In contrast to the earlier compounds, the systemic fungicides not only have translocability in the plant body, but more importantly they have the property of selectively inhibiting specific biochemical reactions of the fungi, somewhat in the manner the antibiotics act on target organisms.

The latest generation of antifungals began to appear in the 1980's. These chemical agents like metalaxyl, are also systemic, but unlike other fungicides they do not exhibit any *in vitro* fungitoxicity. After being absorbed, they are transformed in the plant body into

fungitoxicants. An alternative mode of action of some of these agents is to increase host resistance to the pathogenic fungus. In general, due to their more specific mode of action, all systemic fungicides have a narrower antifungal spectrum than non-systemic fungicides.

The ever-increasing number of different kinds of fungicides and other pesticides, and their widespread use in modern agriculture have aroused concern about their possible interactions with non-target soil organisms which are directly or indirectly involved in the maintenance of soil fertility. This is only one facet of the problem of over-use of pesticides. Due to the recalcitrant nature of many of these compounds, they have a long persistence in nature. While this on one hand provides prolonged protection to crops, on the other hand, reabsorption by plants poses a potential hazard to human and animal health. Another relevant aspect of the problem that has not attracted adequate attention till now is the effects of fungicides on the physico-chemical properties of the soil, like soil colloids, ion-exchange capacity, adsorption, leaching, etc. The results of studies relating to the effect of fungicides on soil organisms or microbiological processes are often highly variable or even conflicting, because in most cases the physico-chemical nature of the soil and the over-all soil environment are not given due consideration. Another important point in the fungicide-microbe interaction studies is that most of them have been conducted under laboratory or glass-house conditions. The results obtained from such experiments may not hold good under field conditions. Moreover, very often pure cultures of organisms are employed for such studies. Their response may not be identical under field conditions, where many different kinds of microorganisms and plants live together. Keeping these limitations in mind, we shall discuss some experimental results relating to interactions between fungicides and microorganisms.

#### *Total microflora*

The effect of pesticides on the total soil microflora can be studied either by counting the numbers of living cells of different kinds of microbes, or by determination of the respiratory oxygen evolution using specific inhibitors. A fungicide would be expected to inhibit some or most fungi present in the soil, without affecting other organisms to any great extent. But the actual situation is often more complex. A fungicide at the beginning may stimulate other organisms by reducing competition for food and space, and also be providing additional organic matter in the form of killed fungi. On the negative side, inhibition of fungal population may result in depletion of certain substrates like phosphates, organic acids and decomposition products of complex substances like lignin which are made available through fungal activities.

In a study with captan and two systemic fungicides, tridemorph and vinclozolin in clayey-loam rice field soil in our laboratory, it was observed that captan exerted an inhibitory influence on the total microflora only at a very high application dose. But the effect was overcome within a period of 4 weeks. Captan had stronger inhibitory effect on actinomycetes than other soil bacteria. On the other hand, both tridemorph and vinclozolin were found to have depressive effect on all biotic components at relatively lower concentrations and the effect became more pronounced with incubation time extending over 8 weeks. Similar observations have been made by other workers, particularly with captan. Naumann (1971) reported that captan applied to a loam soil decreased bacterial count in the 1st week, but thereafter led to increase to 180% of the control, before coming to the

normal level. Agnihotri (1971), Houseworth and Tweedy (1973) and Wainwright and Pugh (1975) also observed a general increase in bacterial numbers. Vinclozolin has been reported by Pommer and Mangold (1975) to have no effect on soil organisms in a humus-sandy soil.

#### *Nitrification*

Oxidation of ammonia released by decomposition of proteins and hydrolysis of urea to nitrate via nitrite is one of the central microbiological processes which has great relevance in crop productivity. The process has been found to be susceptible to many fungicides. In our study with captan, we observed that at a concentration of 10 ppm nitrification was inhibited by 96% till 12 days in an enrichment culture of nitrifying bacteria. Tridemorph was also found to be inhibitory, but at somewhat higher concentrations than captan. Ammonification of urea was also strongly inhibited by tridemorph. The process was less susceptible to captan which inhibited only 14% at 10 ppm concentration. Vinclozolin has not been tested by us, but Pommer and Mangold (loc.cit) reported that it had no effect on nitrification.

#### *Nitrogen fixation*

Another soil microbiological process of great importance is biological nitrogen fixation by asymbiotic and symbiotic organisms. Asymbiotic nitrogen fixation in soil was inhibited by captan only at relatively high concentrations (above 50 ppm). Growth of azotobacters and rhizobia in cultures were inhibited by captan at 200 ppm concentration. Nodulation of pea and mung bean was more susceptible, particularly the number of effective nodules decreased significantly at 50 ppm. In comparison to captan, tridemorph exerted a much stronger inhibitory effect on nodulation. An inhibition of 60 to 70% in nodule number was observed in different grain legumes at 10 ppm concentration of tridemorph. Nodulation was completely inhibited above 50 ppm. The overall growth as indicated by dry weight and heights of the leguminous plants were also depressed by tridemorph, particularly at concentrations above 50 ppm. There is an overall dearth of experimental data regarding the effects of systemic fungicides on legume-rhizobium symbiosis, although it is admitted that due to their translocability within plant body they may profoundly affect the symbiotic process which itself is a very complex interaction.

#### *Effects on enzymes*

As it has been already mentioned, the systemic fungicides in contrast to non-systemic ones exert their effect at specific biochemical target sites. Thus, benzimidazoles are mitotic inhibitors inhibiting tubulin formation. Carboximides inhibit succinate dehydrogenase activity of target organisms. Morpholines which include tridemorph act by inhibition of sterols. But very little is known about how these agents exert inhibition of non-target organisms, if they do at all. Tridemorph is known to inhibit growth of many Gram-positive bacteria which neither synthesise nor require sterols for their membrane. Since dehydrogenases are enzymes which are involved in many biochemical reactions of the respiratory pathway, we investigated the effects of tridemorph on several of these enzymes. It was observed that glucose, pyruvate and succinate dehydrogenase activities in both intact cells as well as in cell-free extracts of a tridemorph sensitive strain of *Rhodococcus* (MIC = 7 ppm) were very strongly inhibited (50% at 6 ppm). In contrast a highly resistant culture of *E. coli*

(MIC = 200 ppm) showed comparatively much less inhibitory effect both with intact cells and extract (15 to 20% at 100 ppm). Thus, the dehydrogenases of sensitive and resistant bacteria differed in their relative susceptibility to tridemorph. It was also found to inhibit the dehydrogenases of germinating mung beans to some extent. The soil dehydrogenase activity was likewise inhibited.

Apart from dehydrogenases, the protease activity of mung bean seedlings was found to decrease when the seeds were pretreated with different concentrations of tridemorph. There was a reduction in the fresh weight of the seedlings and a proportionate increase in protein content, reflecting perhaps the decreased mobilization of proteins due to protease inhibition. However, several other enzymes, like amylase, aldolase, peroxidase etc. remained unaffected by the fungicide.

These results show that fungicide may have adverse effects on useful microorganisms, as well as on plants. Therefore, their over-use both quantitatively and in number of applications to a particular crop may prove to be counterproductive. Dearth of experimental data regarding their non-target effects, particularly with respect to the newly marketed systemic fungicides, points out to the necessity of research in this field under different agronomic and soil conditions.