

SUMMARY

Pesticides are compounds or mixture of compounds used for destroying disease causing agents like insects, pests and pathogens in plants. The pesticides are classified according to their chemical composition, type of target pest(s). There are several advantages of using pesticides, but, the use of pesticides raises a number of environmental concerns. About 98% of the sprayed herbicides and 95% of insecticides reach to non-target species including soil, water and air. Some chlorinated pesticides are concentrated at each level of food chain resulting in 'bio magnification'. Moreover, the so called "beneficial pesticides" also causes severe diseases like cancer, Parkinson, Alzheimer's etc. Therefore, it is necessary to transform/ break down the toxic pesticides to smaller intermediates/substances. There are many physic-chemical processes for degradation. But, these processes are not environment friendly and may result in generating toxic substances. A better and greener method termed as 'bioremediation' is proving a boon for pesticide detoxification. The process involves 'microorganisms or microbes'. Microbes were the first organisms on Earth and paved the way for plants and animals. They are the foundation of biosphere, comprising of 60% of the earths' biomass. The genetic, metabolic and physiologic diversity of microbial species is greater than that present in plants and animals.

The present study exploited the metabolic and physiologic versatility of the microorganisms, specifically bacteria in an attempt to degrade the two toxic pesticides namely 'maneb' which is a fungicide and 'paraquat' which is a herbicide.

The first part of this study contributes in the understanding of sorption processes of maneb and paraquat on the soils having different physic-chemical properties. In case of maneb, the maximum adsorption was seen on clayey soil. Hence, clay content plays an important role in maneb adsorption, only when organic matter is low. The amount of Mn was also found to be increasing with time suggesting the adsorption of Mn along with maneb. The lower desorption rate suggests that the successive crops will be affected by the toxic fungicide. The adsorption process follows Langmuir adsorption isotherm suggesting monolayer adsorption and that the surface has a specific number of sites where the solute molecules can be adsorbed. However, the exact mode of binding and the nature of functional groups involved in the binding process could not be determined. Addition of organic matter to the soils increased retention of the fungicide. This suggests that addition

of organic matter to the soils restricts the mobility of maneb and prevents its pollution to non-target sites. During summers, the aqueous solubility of the fungicide will increase and as a result it will mix with the rain water or river water and will leach off the soils and reach nearby areas, thus causing pollution to non-target sites as well.

Paraquat shows high adsorption/retention (approximately 100% especially at lower concentrations) upon application on soil irrespective of physic-chemical properties. The adsorption process occurs according to Freundlich adsorption isotherm and pseudo second order reaction kinetics. The Freundlich adsorption isotherm is based on the assumptions that adsorption occurs on heterogeneous solid surfaces and by multilayer mechanism. The lower desorption values suggest the ionic bonding between the positively charged paraquat molecules and the negatively charged soil surfaces resulting in chemisorption. No effect of addition of external organic matter was seen on paraquat adsorption. Since the herbicide tends to interact with the soil particles in an irreversible manner, hence, there is possibility of paraquat pollution at the target site(s) and the effect of its application can be seen on the successive crops. The results from the present study would help in designing effective maneb and paraquat application and management strategies in agricultural fields.

The second part of the present study was successful in isolation of different bacterial strains from the surface of an obnoxious weed, namely *Parthenium hysterophorus* growing on sites contaminated with diuron (a herbicide). The isolates comprised of *Firmicutes*, *Proteobacteria* and *Bacteroidates*. One bacterial strain designated as SDS18 was found to be capable of utilising both maneb (upto 150 ppm) and paraquat (upto 200 ppm) as sole source of carbon suggesting the possibility of transformation or degradation of the two pesticides via metabolism. The strain was versatile, since, it utilised EU, ETU (reported intermediates of maneb degradation) and CMP (photolytic product of paraquat) as C sources too. The polyphasic taxonomic data paved the way for complete characterization and identification of the potential bacterial strain SDS18. The strain was belonging to the genus *Pseudomonas* and species *psychrotolerans*. Further experiments revealed the optimum conditions for growth and utilisation of the toxic molecules was in MSM at pH7 and in the presence of ammonium sulphate as N source.

The physiological versatility of the strain SDS18 was exploited for the 'plant growth promoting ability'. Not to add to our surprise, the strain exhibited important plant growth

promoting activities like production of catalase, ammonia, IAA and siderophore. Moreover, the strain showed phosphate solubilisation, ACC and fungicidal activities for *Cladosporium cladosporoides* and *Alternaria citri* too. Hence, it can be concluded that the strain is inimitable in its sense of utilising toxic compounds as an energy source and exhibiting plant growth promoting ability at the same time.

The final part of this study aimed at extraction and identification of probable intermediates of degradation. We were successful in extraction and identification of the probable intermediates of degradation of maneb and paraquat. The updated and robust techniques like HPLC, NMR, FTIR, ESI-MS etc. were employed to find out the structure of extracted intermediates. 2, 3-dihydroxy pyridine was extracted and identified as one of the major product of degradation of paraquat. The formation of such pyridinium compounds in paraquat degradation is not reported yet. In case of maneb, ETU was extracted and identified as the major product. ETU is also reported as the photolytic product of maneb degradation. However, in the present study the carcinogenic ETU was metabolised to smaller compounds entering TCA cycle. Genomic insights of the isolated exquisite strain SDS18 revealed the presence of important genes and enzymes like manganese superoxide dismutase, catalase, superoxide dismutase, peroxidase, paraquat inducible protein etc. with a total of 213 for tolerating the oxidative stress generated by maneb and paraquat. In the category of metabolism of aromatic compounds, presence of genes and enzymes of the subsystem 'catechol branch of beta-ketoadipate pathway', 'homogentisate pathway of aromatic compound degradation', 'N-heterocyclic aromatic compound degradation', 'protocatechuate branch of beta-ketoadipate pathway' etc. were present.

Further studies involving the specific genes and enzymes for degradation could be predicted which could be helpful in the completion of degradation pathway.