6. SUMMARY
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TMTD, a dithiocarbamate, and sevin, a methyl-carbamate had varied effects on soil microflora and soil biological processes such as nitrification, ammonification, nitrogen fixation and plant growth and different soil enzymes like amylase, invertase, dehydrogenase and phosphatase. The effects were time and dose dependent. A concentration of 50 ppm and above was inhibitory for soil bacteria, fungi and actinomycetes. There was an increase (3-10%) in soil population of bacteria and actinomycetes at 10 ppm concentration of pesticides. With increase in incubation time, the soil microflora number tended to recover but the effect was more pronounced at higher pesticide concentrations of 500 and 1000 ppm. The median effective dose (ED₅₀) showed higher values in case of soil microflora. ED₅₀ for TMTD for soil bacteria, fungi and actinomycetes was 2290 ppm, 1140 ppm and 1000 ppm respectively. ED₅₀ values of sevin for soil bacteria, fungi and actinomycetes were 182 ppm, 1060 ppm and 1888 ppm respectively. The high values of ED₅₀ in case of soil microflora revealed that effect of these pesticides on mixed population in soil was short lived and with time the population tend to become normal. Sevin was more effective against soil bacteria than TMTD. Nitrification of diammonium hydrogen phosphate decreased by both TMTD and sevin at all concentrations studied (100, 250 and 500 ppm) from 1st week onwards until the end of incubation time (six weeks). Sevin was more effective against nitrification (ED₅₀=51.88) than TMTD (ED₅₀ = 105.9) or their combination (ED₅₀ = 59.57). At 500 ppm concentration of TMTD or sevin only 17.6 and 5.98 per cent activity remained of that of control.
Ammonification process increased with increase in pesticide concentration as well as incubation time. There was a significant difference between ammonium-nitrogen levels in absence and presence of pesticide at a particular time. The activity enhanced to 125.54 per cent and 125.91 per cent of that of control at 500 ppm levels of TMTD and sevin respectively. TMTD and sevin affected the growth of soybean (Glycine max) plants and also decreased nitrogen fixation in the presence of pesticides. TMTD was more effective against nitrogen fixation ($ED_{50} = 38.11$) than sevin ($ED_{50} = 53.10$) or their combination ($ED_{50} = 64.00$). The results were statistically significant.

Among the soil enzymes; amylase, phosphatase and dehydrogenase were inhibited in the presence of both the pesticides alone and in combination and the activity decreased with increase in pesticide concentration. $ED_{50}$ values of TMTD for amylase, invertase, phosphatase and dehydrogenase were 115.4 ppm, 3981.0 ppm, 430.5 ppm and 707.9 ppm respectively. $ED_{50}$ values of sevin for these enzymes were 891.3 ppm, 912.0 ppm, 302.0 ppm and 549.5 ppm respectively. From $ED_{50}$ values it appeared that amylase was more affected by TMTD whereas sevin inhibited phosphatase the most. Invertase was not affected by TMTD but its activity decreased in presence of sevin. It was also evident from the high value of $ED_{50}$ of TMTD (3981 ppm) for the enzyme invertase as compared to 912 ppm for sevin. The results were shown to be statistically significant.

The organisms degrading TMTD and sevin were isolated from soil. The organism degrading TMTD was identified as Pseudomonas putida and the one degrading sevin was identified as Pseudomonas cepacia. Growth of both the organisms was maximum in nutrient broth in absence
of pesticide but pesticide utilization was maximum in synthetic medium (65% in case of TMTD and 85% in case of sevin) when it was used as a sole source of carbon. The presence of glucose or yeast extract along with glucose decreased utilization of TMTD or sevin by respective organisms. *P. putida* was equally effective in degrading TMTD when inoculated into soil containing the pesticide. It resulted in 50% degradation in just 4 days and only 10% TMTD remained after 16 days. On the basis of growth in the presence of α-ketoglutarate, dimethylamine, methionine and formaldehyde which showed no lag phase and utilization of TMTD, it was observed that these were used as sole source of carbon and nitrogen by *P. putida*. These compounds were suggested as possible degradation products of TMTD and the probable pathway appeared to follow like TMTD → Dimethyldithiocarbamate (DDC) -DDC-α-ketobutyric acid → DMA → Methionine - Formaldehyde + ammonia.

Similarly, *Pseudomonas cepacia* effectively degraded sevin in soil and was quite stable. It could degrade 70% of sevin added in just 4 days and degradation was complete by 16th day when only 1% of sevin remained. The organism was capable of taking up all the possible degradation products of sevin studied without any lag phase i.e. catechol, salicylate, 1-naphtol and 2-napthol. Catechol was readily utilized (100% of catechol taken up in just 5 hours) followed by salicylate (6 hours), 1-napthol and 2-napthol (9 hours).

The enzymatic nature of degradation of both TMTD and sevin was established and the conditions for the production of respective enzymes were optimised. The enzyme produced by *Pseudomonas putida* for the degradation of TMTD was extracellular. The maximum production was
achieved in synthetic medium containing 100 µg TMTD per ml. as sole source of carbon in late log phase (60 h) under shaking conditions at 37°C and pH=7.5 when 2% initial inoculum was used.

The enzyme degrading sevin produced by *Pseudomonas cepacia* was intracellular released after disrupting the cells. The enzyme was maximally produced in synthetic medium containing sevin (200 µg. ml⁻¹) as sole carbon source in stationary phase (72 hours) under shaking conditions at 37°C and pH=7.0.

The enzymes were purified by ammonium sulphate precipitation and cation exchange resin. Final purification was achieved with Sephadex G-200 (17.46 folds of purification for TMTD and 13.36 for sevin). These preparations showed single band on polyacrylamide gel electrophoresis.

The purified enzyme degrading TMTD had a molecular weight of approximately 46,230 daltons. The characterized enzyme had an optimum reaction temperature of 55°C, incubation time of 8 minutes at pH 8.0 in phosphate buffer. The enzyme was completely inhibited (zero activity) by toxic metal salts like mercuric chloride, silver nitrate and stannous chloride and having inhibitory activity in presence of Ferrous chloride (22%), Ferric Chloride (10%), Ferrous sulphate (40%) and Potassium ferrocyanide (75%). Enzyme inhibitors like periodic acid and sodium dithionate completely inhibited the enzyme. The enzyme got denatured at 75°C in just 15' and there was only 8% activity after 5 minutes exposure at 75°C.

The purified enzyme hydrolyzing sevin had a molecular weight of approximately 60,250 daltons. The optimum physico-chemical conditions for the enzyme reaction were; temperature = 50°C; pH = 7.0 (phosphate
buffer); reaction time = 10 minutes and Vmax = 80. Metal salts like stannous chloride and mercuric chloride completely inhibited the enzyme activity. Similarly, there was no activity in presence of enzyme inhibitors like periodic acid and sodium dithionate with varied activity in presence of PCMB (35%), Mercapto ethanol (90%), Hydrogen peroxide (72%) and DDVP (80%). The enzyme degrading sevin was thermolabile and got inactivated at 75°C. There was only 5% activity of the enzyme after exposure to 75°C for 5 minutes.

The sevin degrading organism, *Pseudomonas Cepacia* was found to harbour a plasmid of appox. 42 kb which was responsible for the degradation of pesticide as shown by curing studies with acridine orange. No plasmid was detected in *P.putida* degrading TMTD.

Both the pesticides, TMTD and sevin were found to be non-mutagenic in *Salmonella typhimurium* test of Ames. The purified esterase enzyme was added to sevin resulting in its breakdown and the probable pathway of sevin degradation of *Pseudomonas cepacia* appeared to follow the steps like Sevin → 1-naphthol → 1, 2 - dihydroxynaphthalene → cis-o-hydroxybenzalpyruvate → salicylaldehyde → salicylic acid → catechol - cis, cis-muconate → (+)- muconolactone → β-Ketoadipate- enol lactone → β-ketoadipic acid.

As it was observed that both these pesticides inhibited soil microflora and soil biological processes (nitrification, nitrogen fixation and plant growth) including soil enzymes and it was also established that the respective organisms isolated for the degradation of TMTD and sevin effectively degraded these pesticides in the soil; the organisms were inoculated into soil to study the possible reversal of the effect of these
pesticides on soil biological processes. Pesticide degrading organisms inoculated into soil under nitrification and in presence of pesticides (TMTD, sevin or both) increased NO$_3^-$-N levels and were effective in reversing the inhibitory effect. The NO$_3^-$-N level of 22.08 ppm after 2 weeks of incubation in presence of 250 ppm TMTD was raised to 122.12 in presence of degrading culture which was comparable to control (138.28) without the pesticide or degrading culture. Similarly, nitrogen fixation and plant growth that was drastically reduced by these pesticides got revived in the presence of degrading culture. An activity of 1518 nmoles of C$_2$H$_4$ g$^{-1}$ hr$^{-1}$ in presence of 100 ppm sevin increased to 3254 n moles C$_2$H$_4$ g$^{-1}$ hr$^{-1}$ by addition of sevin degrading culture of *P. cepacia*. The activity of all the soil enzymes studied viz. amylase, invertase, phosphatase and dehydrogenase got revived when the respective pesticide degrading culture was inoculated in the soil.