https://doi.org/10.29321/MAJ.10.A03084

Madras agric. J. 65 (5): 325—328. May, 1978.

Interaction of Two Organophosphorus Pesticides with a *Rhizobium* sp. and Their Degradation *in vitro*

T. SEKAR[1] and A. BALASUBRAMANIAN[2]

Fensulfothion (Dasanit) (0,0-diethyl-0-4 methyl sulphinyl phenyl monothiophosphate) when applied at 2 ppm (lower level) and 5 ppm (recommended level) concentrations to sterilised soil inoculated with the *Rhizobium* sp. (Cowpea group) recorded an increase in the rhizobial numbers for 30 days during incubation and disulfoton (Disyston) (0.0-diethyl-S-2 (ethyl thio) phosphoro dithioate) showed an increase during the first 20 days. Both these chemicals, however, at 10 ppm (higher level) significantly depressed the population of *Rhizobium* in sterilised soil throughout the incubation period. In normal soil, Dasanit applications, at all the three levels, registered an increase in population upto 10 days and a significant reduction thereafter upto 60 days. Disyston also reduced the population of the organism at all the concentrations, the effect being more severe with the higher level. When Fensulfothion and disulfoton were added to th the growth medium *in vitro* and inoculated with the *Rhizobium* sp. 21.4% and 74.5% respectively, of the chemicals were degraded within 7 days.

Several pesticides find their way into soil to which they are repeatedly applied for combating soil borne insect pests and nematodes. The effect of these pesticide residues on soil microbial community has been brought out by several workers (Sreenivasulu and Rangaswami, 1973; Kandasamy *et al*., 1974; Balasubramanian and Gita Nilakantan, 1976). The influence of such residual chemicals on multiplication and survival of the symbiotic nitrogen fixing bacteria, the *Rhizobium* has not been reported so far in any detail. The effect of two soil applied organophosphorus pesticides viz., fensulfothion (Dasanit) (0, 0-dimethyl-0-4-methyl sulphinyl phenyl monothiophosphate) and disulfoton (Disyston) (0,0-diethyl-S-2 (ethyl thio) phosphoro dithioate) on the survival of the *Rhizobium* sp. (cowpea group), in sterilised as well as normal soil, at 2, 5, and 10 ppm levels is presented in this paper.

MATERIALS AND METHODS

Garden soil (pH 7.2, organic carbon 0.692%, total nitrogen 0.07%) sieved to 1 mm size, was filled in glass bottles in 300 g quantities, one set of which was sterilised at 15 p s i for 2 hr and the rest kept unsterilised. Calculated quantities of Dasanit and Disyton were added to both the soils

1 - 2 Instructor and Associate Professor,
Microbiology Laboratory, Department of Biology,
Tamil Nadu Agricultural University, Coimbatore - 641 003.

separately to obtain final concentrations of 2 ppm (lower level), 5 ppm (recommended level) and 10 ppm (higher level) of the active ingredient of the chemicals. A suspension of of the *Rhizobium* sp. containing approximately 1 x 10^{10} cells per ml was inoculated at the rate of 5 ml per bottle and thoroughly mixed. The moisture level of the soil was maintained at 50 per cent of maximum water holding capacity by adding required quantity of sterilised tap water and the bottles ware covered with polyethylene sheets. Appropriate controls were maintained and each treatment was duplicated. Soil samples were drawn initially and periodically thereafter upto 60 days and analysed for the rhizobial population by plate count method (Allen, 1953) using Congo red agar medium) (Mannitol 10.0 g, calcium carbonate 3.0 g, dipotassium hydrogen phosphate 0.5 g, magnesium sulphate 0.2 g, sodium chloride 0.1 g, yeast extract 0.5 g, agar 15.0 g, distilled water 1000 ml and 1:400 aqueous solution of Congo red 10.0 ml/l).

For studying the degradation of the pesticides by the *Rhizobium in vitro* malt extract medium (Iswaran and Jauhri, 1969) with only 0.25 per cent of the carbon source (malt extract) but containing 500 ppm of the technical materials of the pesticides was inoculated with a 72 hr. old culture of *Rhizobium* sp with appropriate controls. After 7 days incubation on a rotary shaker, the cells were harvested by centrifugation and the insecticide residues in the culture filtrate were estimated following the method described by Schumann and Olson (1964).

RESULTS AND DISCUSSION

Effect of the pesticides on survival of the Rhizobium sp.: The results revealed that Dasanit application to sterilised soil recorded a marked reduction in the rhizobial population at all the three levels throughout the incubation period (Table I). In general, Disyston also caused a decline in the rhizobial number, though a stimulation was observed at 2 and 5 ppm levels of the chemical, on 20th day of incubation. In normal soil, however, Dasanit application at all the three levels increased the population of *Rhizobium* on the 10th day of incubation (Table I). These observations are in conformity with the findings of Sreenivasulu and Rangawsami (1973) who recorded a stimulation in the bacterial flora due to application of Dasanit. Disyston imposed a significant inhibitory effect on the population on the 10th day of incubation and the effect was more severe with the higher concentration. In general, the effect of the chemicals on the population of the *Rhizobium* was more pronounced in normal soil which indicated the possibility of more rapid degradation normal (unsterilised) soil than in the sterilised soil.

Degradation of the pesticides by the Rhizobium sp.: The results indicated that both the organophosphorus insecticides were degraded by the *Rhizobium* sp, the maximum percentage degradation being observed in the case of disulfoton (Table II).

TABLE I. Effect of two organophosphorus pesticides survival of the *Rhizobium* sp in sterilized and unsterilized soil (Population in 10⁶/g moisture free soil)

	Incubation period (days)												
Treatment	0	10	20	30	40	50	60	0	10	20	30	40	50	70
Control	34.0	154.2	399.9	206.3	23.9	4.3	2.6	33.3	478.4	163.2	78.3	16.6	5.9	1.7
Dasanit														
2 ppm		108.9	167.6	209.0	31.2	5.9	2.9		646.3	88.8	84.4	8.3	2.3	1.9
5 ppm		93.9	232.5	229.5	19.8	4.0	2.7		562.5	138.7	87.1	8.5	1.7	1.1
10 ppm		64.0	174.2	111.4	17.6	1.7	2.5		757.5	100.6	67.7	7.2	1.9	1.2
Disyston														
2 ppm		164.7	650.0	66.6	11.6	2.6	2.4		190.3	82.3	93.7	15.7	9.8	1.9
5 ppm		147.0	629.3	112.8	11.6	1.6	2.5		225.0	172.4	148.3	13.7	8.3	1.0
10 ppm		66.3	365.5	87.5	7.1	1.1	2.1		156.6	127.7	72.7	12.7	8.7	1.8

C.D (5%) for pesticides : 10.8 C.D (5%) for pestides : 8.1

TABLE II. Degradation of two organophosphorus pesticides by a *Rhizobium* sp.

	*Pesticide residue(ppm)		
Treatment	Uninoculated medium	Inoculated medium	**Pesticide degraded(%)
Fensulfothion	490.3	386.3	21.4
Disulfoton	486.9	123.8	74.5

*Data represent mean of two replications

**Degradation in 7 days

Within a period of 7 days 74.5 per cent of disulfoton and 21.4 per cent of fensulfothion were degraded by the *Rhizobium* sp. A reduction in the original concentration of the insecticides in the uninoculated medium may perhaps, be due to evaporation of the insecticides. Lichtenstein (1972) has demonstrated such an evaporation in several insecticides Mick and Dahm (1970) reported degradation of parathion by *Rhizobium japonicum* and *R. meliloti*. Another organophosphorus compound, malathion has been found to be metabolised by *R. leguminosarum* and *R.trifolii* (Mostafa *et al.*, 1972). In the present case also, considerable quantities of the pesticides were metabolised and it is quite possible that the degraded products were utilised by the *Rhizobium* sp. Sekar (1977) has demonstrated that the *Rhizobium* sp. utilised disulfoton *in vitro* as its sole carbon, phosphorus and sulphur source. In soil environment also, such degradation and utilisation of the pesticidal molecules by the *Rhizobium* sp. can be expected.

The authors acknowledge the financial assistance provided by the International Atomic Energy Agency, Vienna, Austria, in the form of a Junior Fellowship to the first author under a research contract (RC 1660/IND).

REFERENCES

ALLEN, O.N. 1953. *Experiments in Soil Bacteriology*. Burgess Publishing Co., Minneapolis. U.S.A. pp. 69-70.

BALASUBRMANIAN, A. and GITA NILAKANTAN. 1976. Effect of Temik on the microflora and indole acetic acid production of a red sandy loam soil. *Curr. Res.* 5 : 15-17.

ISWARAN, V. and K.S. JAUHRI. 1969. Malt extract as a good source of carbon for large scale production of *Rhizobium* inoculants. *Curr. Sci.* 38 : 67.

KANDASAMY, D., R. BHASKARAN, G. OBLISAMI and T. R. SUBRAMANIAN. 1974. Changes in the rhizosphere microflora of bhendi as influenced by application of disyston. *Madras agric. J.* 61 : 10-12.

LICHTENSTEIN, E. P. 1972. Environmental factors affecting fate of pesticides. In: *Degradation of synthetic organic molecules in the biosphere. Nat. Acad. Sciences.* pp. 190-205.

MICK, P.A. and P.A. DAHM. 1970. Metabolism of parathion by two species of *Rhizobium*. *J. Econ. Entomol.* 63 : 1155-59.

MOSTOFA, Y.I., I.M.I. FAKHR., M.R.E. BAHIG and Y. A. El-ZAWAHRY. 1972. Metabolism of organophosphorus insecticides XIII. Degradation of malathion by *Rhizobium* spp. *Arch. Mikrobiol.* 86 : 221-24.

SCHUMANN, S. and T. OLSON. 1964. Colorimetric determination of disyston residues in soil. *Report No. 13059 of Chemagro Corporation Res. Dept.*

SEKAR, T. 1977. *Effect of Pesticides on the growth, metabolism and symbiosis of a Rhizobium* sp. unpub.M.Sc. Thesis submitted to Tamil Nadu Agri. University, Coimbatore

SREENIVASULU, S. and G. RANGASWAMI. 1973. Studies on the effect of granular organophosphorus insecticides on soil microflora. *Indian J. Microbiol.* 13 : 89-95.