Metabolism of Organophosphorus Insecticides
XIII. Degradation of Malathion by *Rhizobium* spp.


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**Summary.** The metabolism of $^{32}$P-Malathion in *Rhizobium leguminosarum* and *Rhizobium trifolii* has been investigated. In addition to inorganic phosphates and/or thiophosphates, 5 hydrolytic metabolites could be identified. The carboxylic acid derivatives constituted the major portion ($35-40\%$ of the total metabolites output) suggesting the presence of powerful carboxyesterases in both *Rhizobium* spp. Malaoxon could not be detected in the media of both organisms.

Malathion (0,0-dimethyl S-[1,2-bis-carbethoxy]ethyl phosphorodithioate), is an important and widely used pesticide. Its degradation in insects (Bigley and Plapp, 1962; Matsumura and Dauterman, 1964; Matsumura and Voss, 1964, 1965), animals (Krueger and O’Brien, 1956; March *et al.*, 1956; Seume and O’Brien, 1960) and plants (Bourke *et al.*, 1968) have been well catalogued. In soil fungus, *Trichoderma viride*, Malathion was apparently degraded via two routes that did not include the oxidation product Malaoxon (Matsumura and Boush, 1966). The presence of powerful carboxyesterases was suggested according to the fact that the carboxylic acid derivatives of Malathion constituted the major portion of the metabolites. Some variants of *T. viride* also showed high demethylation activity.

In Egypt Malathion has been accepted for controlling pests on vegetables, field crops, fruits and ornamentals.

The present work was undertaken to gain insight into the mode of action and degradation of $^{32}$P-Malathion by *Rhizobium trifolii* and *Rhizobium leguminosarum*, the two common species of *Rhizobium* in Egyptian soil.

$^{32}$P-Labelled Malathion was prepared according to Zayed *et al.*, (1972) and was demonstrated to be chromatographically pure.

Into a 50 ml sterile Allison’s medium containing Na NO$_3$ as nitrogen source and 5 mg of labelled Malathion (specific activity 0.025 mC/mg), 5 ml of 5 days-old culture of either *Rhizobium trifolii* or *Rhizobium leguminosarum* were inoculated. The pH of the medium was adjusted at 6.0 using 1% of H$_3$PO$_4$ solution (Wilson and Knight, 1952) to avoid any possible hydrolysis of the toxicant. In solution
or in a finely emulsified form in aqueous or other liquid medium, Malathion was found to be quite stable over the pH range 2 to 7. According to Koivistoinen and Aalto (1970), the hydrolysis rate constant (K) under the present conditions is $<10^{-3}$. For sake of comparison, control flasks were prepared free from bacteria but with all conditions maintained. After incubation for one week at 30°C, the reaction mixtures were centrifuged and the clear media were then extracted 5 times with chloroform. The final pH at the end of the experiment remained unchanged due to the presence of the two phosphate salts, $K_2HPO_4$ and $KH_2PO_4$, as constituents of Allison's medium. The radioactivity of the combined chloroform extracts as well as the aqueous fractions was measured by G. M. counter. Following evaporation to a known volume, both aqueous and chloroform fractions were analyzed by paper chromatography using acetonitrile—water—ammonia (85:14:1) and isopropanol—water—ammonia (75:1:24) systems (Zayed et al., 1972). The chromatograms were assayed radiometrically using a Frieske & Hoepfner radioscanner. Different spots were made visible by spraying with Hanes-Isherwood reagent (Hanes and Isherwood, 1949). Phosphorothioate compounds were located by spraying the chromatograms with 2% cupric chloride solution, and then with 0.50% potassium ferricyanide solution where red-brown spots on a yellow-green background were obtained (Plapp and Casida, 1958).

Chloroform fraction of *Rhizobium leguminosarum* was found to contain only unchanged Malathion which amounted to 11% of the applied dose. In case of *Rhizobium trifolii*, however, a very small amount (about 0.5% of Malaoxon was detected in addition to the unchanged Malathion which accounted only for 4.5% of the initial applied activity.

Radiomasurements of the aqueous fractions showed that about 87% and 67% of the applied dose was metabolized during the incubation period with *Rhizobium trifolii* and *Rhizobium leguminosarum* respectively.

Chromatographic analysis of the hydrolytic products revealed the presence of at least five metabolites in addition to inorganic phosphates and/or thiophosphates which were clearly shown by the presence of a notable spot at the base line. This spot is believed to be more than one substance because of its chromatographic behavior (Zayed et al., 1972) and may represent secondary cleavage of one or more of the primary hydrolytic metabolites. The identified water soluble metabolites are shown in Table 1.

The products of metabolism indicate that *Rhizobium* spp. are very active in degrading Malathion through carboxylesteratic hydrolysis as well as by desmethylation process. The initially formed products may suffer further degradation leading to a variety of hydrolytic substances; the final product being inorganic phosphate and/or thiophosphate. The carboxylic acid derivatives of Malathion constitute the major portion of metabolites suggesting the presence of powerful carboxyesterases in the *Rhizobium* spp. However, carboxyesterase was unable to hydrolyze the remaining carbethoxy group when Malathion monoaicid was added to the enzyme in a special system (Menzer and Dauterman, 1970). Thus the resulting Malathion diacid which represents a relatively high per-