

METABOLISM OF SOME PESTICIDES
BY SOIL MICROORGANISMS

By

Talaat Mohammed El-Hoseiny

B.Sc., (1959)
M.Sc., (1965)



Thesis Submitted in Partial Fulfilment
of the Requirements for
DOCTOR OF PHILOSOPHY

2871

in
Insecticides



Plant Protection Dept.
Fac. of Agriculture
Ain Shams University

1969

This Thesis has been
Approved by :

Suleh A. K.

Harmond Teed

M. R. Halligan

Date : / / 1969

...00000...

A C K N O W L E D G M E N T

This work has been carried out in the Faculty of Agric., Ain Shams Univ. under the supervision of Dr. M.M. Zaki, Prof. of Insecticides, Dr. M.R. Abu-El-Ghar, Prof. of Insecticides, Plant Protection Dept., and Dr. S.A.Z. Mahmoud, Prof. of Bacteriology, Agric. Botany Dept. The writer is indebted to them for suggesting the problem , supervision, keeping interest, progressive criticism and encouragement without which this work could not be performed.

...ooOoo...

C O N T E N T S

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	3
MATERIALS AND METHODS	20
Insecticides Used	20
1. Delnav	20
2. Dimethoate	20
3. Thiocron	21
4. Temic	21
1. Soil microbiol flora and insecticidal residues under successive applications of insecticides	21
II. Isolation of the resistant and adapted microorganisms	23
III. Efficiency of isolates in insecticidal degradation	24
IV. Metabolism of the insecticides by soil microorganisms in pure cultures	25
V. Metabolism of Temic by active strains of soil microorganisms	26
VI. Degradation of insecticides in soils inoculated with active organisms	27
Chemical Determinations	30
I. Extraction of insecticides from soil	30
II. Determination of insecticidal residues ..	31
III. Enzymatic determinations	32
IV. Chromatographic studies	34
RESULTS AND DISCUSSION	39
I. Effect of insecticides on total count ...	39
A. Effect of successive application at the normal rate	39
B. Effect of gradually increasing concentrations	41

II. Residues of insecticides under repeated applications	45
III. Anticholinesterase activity in soil repeatedly treated with insecticides	57
IV. Microbial changes due to repeated application of toxicants	64
V. Isolation of active organisms degrading insecticides	75
VI. Metabolism of insecticides by pure cultures	79
1. Degradation of insecticides with the most efficient strains	79
2. Anticholinesterase activity in pure cultures treated with the insecticides	88
3. Metabolism of insecticides by pure cultures of active organisms	97
VII. Metabolism of insecticides in soil	116
1. Degradation of insecticides in soil inoculated with active organisms	116
2. Metabolic byproducts of insecticides in soil	129
SUMMARY	151
REFERENCES	160
ARABIC SUMMARY	

...ooOoo...

It is well known that soil microorganisms play a major role in the mineralization of organic matter in the soil and in supplying plants with available nutrients. As inhibitors for biological systems, pesticides may exert simultaneously a deleterious effect upon the saprophytic soil population. Numerous groups of microorganisms, on the other hand, can after a lag period, adapt themselves to decompose these toxic chemicals. Several strains of bacteria, yeast, and algae were isolated which can grow on different pesticides with the formation of various metabolites. Most insecticides, subject to microbial attack, are ultimately degraded to inactive products like DDT and Toxaphene; converted to toxic products with greater persistence than the parent compounds like Aldrin and Dieldrin or decomposed to yield numerous metabolites, many of which are synergistic among themselves and with parent compounds like Dimethoate and Phorate (Whitney, 1967).

The aim of this study was to investigate the metabolism of some pesticides, extensively used in Egypt, by soil microorganisms. A great deal of work has been devoted to the isolation and identification of several bacterial strains that can degrade the used pesticides. The study was also focused on the metabolic byproducts of these pesticides released by the active isolates in both pure culture and soil.

REVIEW OF LITERATURE

I. Insecticidal Effects of Toxicants :

Dimethoate :

All the introduced formulations of Dimethoate were applied to soil by several investigators to combat numerous insects. Besides their foliage spraying, Dimethoate emulsions were used as soil drench, sprayed to the ground, applied parallel to the seed furrow, or seed pieces dipping to control : potato tuberworm, two-spotted spider mite, Lygus hesperus, potato aphids, green peach aphid, alfalfa seed chalcid, Bactra verutana (stem borer), thrips, and the Hemlock fiorinia scale (Bacon, 1960; Wilcox and Howland, 1960; Koehler, 1963; Pond, 1963; Shorey, 1963; Bacon et al., 1964; Jefferson and Humphery, 1964; Jefferson et al., 1964 and John et al., 1964).

Dimethoate granules were used as side-dressing, seed bed treatment, usual mixing with the soil surface, and applied parallel to the seed furrow. Shread (1960), Boush et al. (1963), Tappan (1963) and Jefferson et al. (1964) applied Dimethoate granules to the soil surface by usual mixing against scale insects, leaf miners, lace bugs, southern corn rootworm on peanuts, green peach aphid, and thrips. Wilcox and Howland (1960) applied

Thiocron granules formulation at a rate of 1.5 lb. / acre. Selim (1967) applied Thiocron emulsions to broad bean plants. He provided evidence of translocation of Thiocron through the vascular systems by both bioassay and chemical analysis tests.

Thiocron emulsions were found by many investigators to combat the potato leafhopper, Tetranychus cinnabarinus and alfalfa weevil (Wressell and Driscoll, 1964; El-defrawi et al., 1965 and Armbrust and Gyrisco, 1966).

Delnav :

Several investigators stated that Delnav effectively suppressed rust mite in citrus trees, potato leaf hopper on alfalfa, brown cotton leaf worm, Tetranychus orlanticus, Alabama ergillacae, Metateranychus citri , Vastes fockeui, Tetranychus telarius, Epilachna varivestis and Tetranychus cinnabarinus. (Leiderman, 1950; Curtis and Cleveland, 1955; Lloyd and Martin, 1956; Robertson and Arant, 1956; Anthan, 1957; Jeppson et al., 1957; Adkisson, 1958; Attiah, 1958; Brett and Brubober, 1958; and El-Defrawi et al., 1965; with respect to the previous pests). Mulla (1960) used Delnav emulsions as soil treatment against the Eye Gnat, Hippelates collusor. Bartlett (1963) stated that Delnav as a 25% wettable powder formulation was highly toxic to 5 species of parasitic hymenopterans and 3 species of predatory coccinellids,

Temio

Harris and Mazurek (1966) evaluated some candidate materials as potential soil insecticides. They found that SC 21149 (Temio) is an excellent soil insecticide. They stated that several materials, including some of the most commonly used soil insecticides showed good activity in moist mineral soil but were strongly inactivated in both dry mineral soil and moist muck soil.

Although Temio is a new compound in the field of application, extensive investigations were made concerning its toxicity. Many investigators applied Temio to soil directly, in the furrow at planting time or in the fertilizer band, and gave almost complete control of birch leaf miner (for 2 years), holly leaf miner, Andromeda lace bug, cotton thrips and aphid, serpentine leaf miner, potato psyllid, green peach aphid, tobacco thrips, caged boll weevils, two spotted spider mite and tarnished plant bug (Watson, 1965; Davis et al., 1966; Gerhardt, 1966; Perimmer, 1966 and Shread, 1966).

Baranowski (1966) added Temio to the nutrient solution of hydroponically grown roses. Temio provided control of mites without phytotoxicity. However, Boling and HacsKaylo (1966) observed slight or no marginal burning on cotton seedlings treated with 10% granulated Temio

at the rate of 1-5 lb./acre. Cooper and House (1967) stated that 20 - 200 ppm of Temic gave nearly complete control of powdery mildew in new apple foliage whereas doses more than 200 ppm were phytotoxic.

II. Degradation of Pesticides in Soil :

Unlike some of their inorganic predecessors, organic pesticides are decomposed by biological and physicochemical processes which influence the amount that will be found in the environment (Mitchell, 1967). Generally, ^{there} ~~their~~ are two contradicting groups of investigators concerning whether the degradation of pesticides in soil is a biological process or due to the physico-chemical properties of soil.

The first group of investigators thiorized that the basic factor involved in the interaction between insecticides and mineral soil is the physical phenomenon of adsorption. Harris (1964) stated that the toxicity of the insecticide, to an extent, is inversely proportional to the degree to which the insecticide is adsorbed by the soil.

The phenomenon of insecticide sorption by soil was first recognized by Hadaway and Barlow (1951) and Downs et al. (1951) who established that solid particles of

It was subsequently shown by Baralow and Hadaway (1956 a) that the loss of insecticide activity on mud surfaces occurs by a physical mechanism: adsorption. It is general for insecticides examined, and is not restricted to those of one particular group with similar chemical properties.

In other studies it has been established that the degree of insecticide adsorption is reduced under conditions of high relative humidity (Wade, 1955; Baralow and Hadaway, 1956 b; and Gerolt, 1961). Baralow and Hadaway (1956 b) theorized that their results could be explained on the basis of competition between the water and the insecticides. The toxicity of an insecticide in moist soil will be dependent on both the affinity of the soil particles for that specific insecticide and to the ability of the insecticide to compete with water molecules for the active sites on the soil particles. Bohn (1964) studied the decomposition of Dimethoate following its application to a cultivated sandy loam soil at the rate of 1 lb.actual /acre of an EC formulation. Half of the insecticide in the upper 3 inches of soil disappeared in 4 days in drought conditions and 2.5 days after moderate rainfall. On the other hand, Bacon et al (1964) stated that Dimethoate was effective when applied to wet soil while it was not

effective when applied to dry soil. Also, Harris (1961) found that Diazinon was less toxic in dry soil (94 relative humidity) than in wet soil (124 R.H.) in the order of 134.6 times. He suggested that the effect of soil moisture on toxicity should be taken into consideration when outlining control recommendations.

The soil type, percentage of organic matter, pH and other properties of soil have profound influences on the rate of pesticides degradation in the soil as has been stated by several authors. Getzin and Chapman(1960) determined the persistence and the degree of binding of Phorate in three soils and quartz sand. Phorate was lost rapidly from quartz sand than the three soils. Zaki and Reynolds (1961) reported more rapid toxicant uptake by plants from sandy soils than from clay soils. They also reported more rapid loss through leaching in sandy soil. Leigh (1963) stated that sidedressing application of Dimethoate controlled spider mites in some locations but failed in the others. This failure appeared to relate to soil type.

Beran and Guth (1966) found that persistence of Parathion increased in soils having a high pH as well as a low silt and clay content. Parathion also showed less persistence in soils high in humus content. Getzin and

rosefield (1966) studied the persistence of 14 C-labeled Diazinon and Zinophos in four soils under laboratory conditions at 25°C. The disappearance rate of C^{14} -Diazinon was similar in the 4 soils while the persistence of C^{14} -Zinophos varied considerably in the 4 soils. The time required for 50% loss in an organic, sandy loam, silt loam, and clay loam soils was approximately, 10, 6, 4 and 1.5 weeks respectively. Harris (1966) stated that, in moist soils, the bioactivity of the insecticides was dependent on the organic content of the soil. Heptachlor was less active by a factor of 204, Diazinon by 283, Nemacide by 546, DDT by 965 and Parathion by 1137 in muck soil than in quartz sand. Bryce (1967) found that the adsorption of Disulfoton by 17 types of soil was closely related to the amount of organic matter in the soil.

The second group of investigators stated that almost complete inactivation of the insecticides occurs in soils containing active microbial population. The insecticides persist for longer periods in soils of low number of microorganisms or of low microbial activity. Neumann (1959) stated that Parathion was rapidly decomposed by bacteria in soil and stimulated the increase of various physiological groups. Lichtenstein and Schulz (1960) stated that Aldrin and Heptachlor were epoxidized by microorganisms to form toxic products with greater persistence than the parent

compounds. Dewey et al. (1965) reported that the persistence of Trithion increased significantly in soil which was autoclaved or fumigated with vapam. The longer persistence in such soil apparently resulted from a partial destruction of the soil microorganisms. El-Hoseiny (1964) found that the rate of decomposition of DDT, Toxaphene, Sevin, Di-syston and Meta-iso-systox in nonsterile soil was apparently higher than in sterile soil. Lichtenstein and Schulz (1964) reported that the degradation of Parathion was either by hydrolysis or by reduction to its amino form depending on population of soil microorganisms. Dewey and Parker (1965) and Parker and Dewey (1965) stated that Dimethoate and Phorate when applied to soil are attacked by microorganisms and yield numerous metabolites, many of which are synergistic among themselves and with parent material. Hall and Sun (1965) stated that Bidrin was quickly detoxified in soil. However, considerable stabilization of Bidrin in soil was observed when treated with 500 ppm of several fungicides and antibiotics. Detoxication of Bidrin was also greatly reduced in sterilized or air dry soil. Yasuno et al. (1966) studied the inactivation of Fenitrothion, Parathion, and Methyl parathion by bacteria in polluted water. They found that the inactivation was associated with the presence of living bacterial cells, since no signs of the decomposition