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THE EFFECTS OF INSECTICIDE ENDRIN ON SOYBEAN ROOT NODULES BACTERIA *RHIZOBIUM JAPONICUM*[*)]

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R I N G K A S A N

Telah diselidiki pèngaruh insektisida Endrin terhadap bakteri nodula akar kacang kedele Rhizobium japonicum. Ternyata dalam percobaan dengan menggunakan pot yang disertai penambahan Endrin, tidak memberikan perubahan jumlah dari bakteri tersebut selama pertumbuhan kacang kedele. Strains Rhizobium japonicum dapat dipisahkan dari tanah-tanah yang diberi perlakuan Endrin. Biakan-biakan dari bakteri tersebut tumbuh baik pada media yang diberi penambahan Endrin sebagai sumber C.

A B S T R A C T

The effects of Endrin on soybean root nodules bacteria Rhizobium japonicum has been studied. In pot experiments with soybean and addition of Endrin gave no quantitative change of these bacteria during any part of the vegetation period. Rhizobium japonicum strains have been isolated from Endrin treated soil. These cultures grew fairly well in medium with Endrin as the sole source of carbon.

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INTRODUCTION

Recently introduced pesticides designed for the control of insects are demanding more and more attention to study the effects of these chemicals on soil microorganisms (Alexander, 1969 and Gillberg, 1971). Wilson and Choudri (1946) observed that DDT at less than 0.1% did not injure nitrifiers, ammonifiers, nitrogen fixers or sulfur oxidizing microorganisms. Benzene Hexachloride (BHC) and Chlordane were found injurious to nitrifiers at concentration above 0.5% (Jones, 1952).

Although there are no published data concerned specifically with microbial effects of Endrin (1, 2, 3, 4, 10, 10-Hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-endo-1, 4-endo-5, 8- Dimethanonaphthalene) to soil bacteria in Indonesia, glasshouse and laboratory studies were undertaken to determine its microbial effects, especially on soybean root nodules bacteria *Rhizobium japonicum*.

MATERIALS AND METHODS

Two different types of experiments were performed.
(1) Determination of the total number of *Rhizobium japonicum* in Endrin-treated soils.

The soil samples were taken from a pot experiment in glasshouse condition. In this experiment, soybean plants were grown in pots with sandy soil, and inoculated with *Rhizobium japonicum* Str. IU-221 ex-Cirebon. Further, graded amounts of Endrin were added: 25, 50, 75, 100, 125 and 150 ppm. Four replicate pots were set up. They were watered once or twice daily depending on the weather. The soil sampling was done with soil auger, by means of which a 1-cm thick column of the whole profile was taken from each pot without disturbing the plants. At each determination 2-3 gr of fresh soil was carefully weighed in sterile petri plate. Then the soil was finely suspended in 200 ml sterile aquadest in a Waring-blendor for 30 seconds. From this suspension, 10 ml was pipetted to 90 ml of sterile aquadest and, after shaking, serial dilutions were made with YM-agar medium (Vincent, 1970): 0.5 gr K_2HPO_4, 0.2 gr $MgSO_4.7H_20$, 0.1 gr NaCl, 10 gr mannitol, 0.4 gr yeast extract and 1.000 ml aquadest. Four replicate plates were set up from three consecutive dilutions. Plates were counted after 2-4 days at 24° $\pm$ 2°C.
(2) Isolation of Endrin - adapting *Rhizobium japonicum* from these soils and a study of their effect on Endrin in pure culture. Isolated *Rhizobium japonicum* from (1) were culture in

Isolated *Rhizobium japonicum* from (1) were cultured in YM-agar and YM-broth (Vincent, 1970), and 5 ppm of Endrin was added. After 3 days of incubation at 24° $\pm$ 2°C., the Endrin concentration was increased to at least 50 ppm and the manni-

tol concent was decreased to at least 1 gr. Direct experiments without the initial-low concentration of Endrin were also started. Following this enrichment of Endrin-adapting *Rhizobium japonicum*, agar plates containing YM - agar medium were streaked with loops from the solution.

RESULTS AND DISCUSSION

As is seen from Table I., the Endrin-treated soils in no case show significant differences from the untreated (control) ones as to the numbers of bacteria. The growth of *Rhizobium japonicum* cultures were examined on YM-medium in control series and in series with 5 ppm to at least 50 ppm, no difference in development could be observed. All cultures grew fairly well in liquid or on solid medium with Endrin as the sole source of carbon.

On the basis of these experiments it is believed that Endrin at rates as high as 100 ppm, excessive from the standpoint of recommended application, showed increased *Rhizobium japonicum* population.

Fletcher and Bollen (1954) stated that Aldrin at 200 and 1.000 ppm had a definite stimulatory influence upon the total number of bacteria in soils. On the basis of a 2.000.000 pound acre these treatments are equivalent to application of 400 and 2.000 pounds per acre respectively. Although normal application rates rarely exceed 5 pounds per acre.

There are two general ways in which an interaction can occur between pesticides and microorganisms (Alexander, 1969). A first interaction, the chemicals that come into soil deliberately are designed to act on a limited or broad spectrum of microorganisms, and it is not unreasonable to assume that some of the antimicro-biological principles may like-wise affect the saprophytic microorganisms, and the result of which might be detrimental rather than beneficial to plant growth and crop yield. A second interaction must also be considered. As most pesticides are organic, they may conceivably serve as substrates to support the growth of small or large segments of the bacteria. Proliferation of these selected species would in turn lead to the decomposition of the component and its ultimate disappearance from the environment.

The results of these experiments confirm the hypotheses of Alexander (1969), Fletcher and Bollen (1954) and Jones (1952) that the increased tolerance is due to genetic factors. This genetical inhomogenecity among the rhizobial bacteria, points on a possibility to adapt these bacteria to human needs by devising suitable screening programs, into which selection factors could very well be introduced.

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Table I.: Numbers of *Rhizobium japonicum* in Endrin - treated soils with soybean in pot experiments.

Days after addition of Endrin	Endrin (ppm)	*Rhizobium japonicum* per gram of dry soils ($x10^3$)
5	0	1.2×10^4
5	25	1.4×10^4
5	50	1.1×10^4
5	75	1.2×10^4
5	100	1.2×10^4
5	125	3.7×10^2
5	150	1.8×10^2
10	0	2.9×10^4
10	25	8.1×10^4
10	50	4.7×10^3
10	75	2.9×10^4
10	100	8.3×10^4
10	125	2.1×10^2
10	150	1.3×10^2
15	0	1.8×10^5
15	25	6.1×10^5

Days after addition of Endrin	Endrin (ppm)	*Rhizobium japonicum* per gram of dry soils ($x10^3$)
15	50	4.2×10^5
15	75	9.3×10^5
15	100	6.7×10^5
15	125	6.5×10^2
15	150	3.9×10^2
20	0	1.2×10^6
20	25	4.9×10^6
20	50	1.8×10^6
20	75	2.9×10^6
20	100	4.7×10^6
20	125	2.2×10^2
20	150	1.1×10^2
25	0	8.1×10^6
25	25	5.6×10^7
25	50	9.3×10^7
25	75	9.4×10^7
25	100	6.9×10^7
25	125	6.3×10
25	150	3.9×10
30	0	9.6×10^4
30	25	8.9×10^7

Days after addition of Endrin	Endrin (ppm)	*Rhizobium japonicum* per gram of dry soils ($x10^3$)
30	50	7.4×10^7
30	75	9.1×10^7
30	100	5.5×10^7
30	125	4.6 x 10
30	150	2.1 x 10

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