日植病報 35: 347-350 (1969)

Ann. Phytopath. Soc. Japan 35: 347-350 (1969)

Absorption of γ -BHC by *Fusarium roseum* Link in Culture*

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上山昭則**・江川 宏**・津田盛也**: 培地に加えた γ -BHC の *Fusarium roseum* による吸収

Although γ -BHC (γ -1, 2, 3, 4, 5, 6-hexachlorocyclohexane) is being used widely as an insecticide, information on the microbial degradation of γ -BHC is scanty.¹⁾ Raghu and MacRae³⁾ reported a possibility of the biodegradation of γ -BHC in flooded paddy soil, but they did not attempt to isolate the causal micro-organisms.

In the course of our study on the microbial degradation of γ -BHC, it was found that a considerable amount of it was absorbed (or adsorbed) onto the fungal mycelium from the culturing medium.

As a first step in studying the biochemical transformation of γ -BHC by micro-organisms in soil, a sterile assay system was planned. γ -BHC was quantitatively determined by a Yanagimoto gas-liquid chromatograph (GLC) (Type 5DH) with an electron capture detector on a SE-column (2.25 m) at 190°C. The concentration of γ -BHC in *n*-hexane solution was proved to relate linearly to peak height in the GLC in the range of 10 to 100 μ g.

A modified Czapek-KH₂PO₄ medium containing γ -BHC at a concentration of 0.1 μ g/ml was used for culturing fungi. This medium was previously shown to be favourable for determining γ -BHC remaining in the medium after autoclaving at 120°C, since ordinary Czapek medium containing K₂HPO₄ accelerated degradation of γ -BHC under these conditions. After autoclaving the media, the following five fungi were inoculated onto them and cultured at 28°C: *Aspergillus oryzae*, *Cladosporium herbarum*, *Fusarium roseum*, *Helminthosporium oryzae* and *Neurospora crassa*.

After culturing for 2, 4, 6, 8 and 12 days, γ -BHC was extracted from the medium with *n*-hexane, dried over anhydrous sodium sulphate, and then subjected to GLC analysis under the conditions mentioned above. Blank tests (without fungi) were also carried out. The decreasing percentage of γ -BHC in the medium was calculated by the following formula: γ -BHC decreased = $100 - (h/H) \times 100(\%)$, where (h) is the peak height of the inoculated medium and (H) is the peak height of non-inoculated one.

The analytical results of the *n*-hexane extracts showed that only 30-40% of γ -BHC remained in the medium after 4 days and only 10-30% after 8 days incubation (Fig. 1).

To clarify the mechanism for decrease of γ -BHC in the medium in more detail, *F. roseum* was selected for further experiments: the mycelial mat, which was previously cultured on a modified Czapek-KH₂PO₄ medium (without γ -BHC), was washed thoroughly with deionized water. Then 200 mg of the fresh mycelium was added to the Czapek-KH₂PO₄- γ -BHC medium (4 ml).

* Studies on the biodegradation of pesticides by micro-organisms (2). This paper was read at the Meeting of the Kansai Division of the Phytopathological Society of Japan held in Kochi on Nov. 21-22, 1968.

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Fig. 1. Decrease of γ -BHC in the medium inoculated with several fungi.

- A. *Fusarium roseum* (SU-G)
- B. *Aspergillus oryzae* IFO-4405
- C. *Helminthosporium oryzae* (KU-13)
- D. *Cladosporium herbarum* IFO-6538
- E. *Neurospora crassa* IFO-6067

Fig. 2. Relation of incubation time of the mycelium of *Fusarium roseum* to the decrease of γ -BHC in the medium.

The mixture was shaken for a period of 1/2, 1, 2, 3, 4, 5 and 6 hours. All tubes, without relation to shaking period, gave 80-90% decrease of γ -BHC in the extracts (Fig. 2).

To determine the relation between the mycelial weight and decrease of γ -BHC concentration in the medium, decreasing percentage of γ -BHC in the *n*-hexane extract was measured by using increasing amounts of fresh mycelium from 10 to 200 mg with one hour shaking at 30°C. The quantity of γ -BHC in the *n*-hexane extract decreased with increase in mycelial weight within the range of this experiment (Fig. 3).

To determine the effect of dead mycelium on the decrease of γ -BHC in the *n*-hexane extract from the medium, 200 mg lots of mycelium were subjected to the following pre-treatments: 1) in 70% EtOH, 10 min., 2) in boiling water, 10 min., 3) in 0.1% HgCl₂, 10 min., and 4) in formaldehyde gas, 10 min. Then the mycelium was inoculated on a modified Czapek-KH₂PO₄- γ -

Fig. 3. Relation of fresh mycelial weight of *Fusarium roseum* to decrease of γ -BHC in the medium.

Culture medium	γ -BHC detected		
	Sample-1	Sample-2	Sample-3
Czapek- KH_2PO_4 - γ -BHC medium	+	+	+
Czapek- KH_2PO_4 medium	-	-	-

Fig. 4. Detection of γ -BHC from the mycelia of *Fusarium roseum* cultured on a modified Czapek- KH_2PO_4 - γ -BHC medium.

BHC medium. After shaking for one hour at 30°C, the quantity of γ -BHC in the *n*-hexane extract from the medium was determined. In every pre-treatment, only 10-30% of γ -BHC was found in the extract.

Recently, Ko and Lockwood²⁾ reported that uptake of dieldrin by mycelium of *Rhizoctonia solani* was the same with living or dead mycelium. We obtained the same tendency on γ -BHC.

In further experiments with living mycelium, the peak of γ -BHC was detectable in the *n*-hexane extract of mycelium, after it had been filtered, washed thoroughly with deionized water, and then crushed with a mortar (Fig. 4).

Judging from these results, it is evident that *F. roseum* may absorb (or adsorb) γ -BHC onto the fungal mycelium whether it is alive or dead. Adsorption of γ -BHC by fungi may be an important factor in the retention of this compound in soil.

Further studies on the fate of γ -BHC after absorption onto the fungal mycelium are now in progress.

Acknowledgement: We are indebted to Dr. S. Ishii, Professor of Kyoto University, for the discussion of the problem, and to Dr. T. O. Browning, Professor of University of Adelaide, Australia, for reading the draft. Thanks are due to Dr. T. Taniguchi, Nippon Soda Co. Ltd., Tokyo for kindly supplying BHC-isomers. (Received February 1, 1969)

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和文摘要

培地に加えた γ -BHC の *Fusarium roseum* による吸収

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γ -BHC 加用培地にカビを接種し振とう培養すると、4-8 日後には培地中の γ -BHC の減少率は 60-80% であった。この現象は *Neurospora crassa* ほか 4 種のカビで確認された。つぎに *Fusarium roseum* を用いてさらにくわしく検討した。あらかじめ γ -BHC 無添加培地に生育させた菌体を水洗し 200 mg ずつ γ -BHC 加用培地に加えて 30 分-6 時間振とうした。その結果 γ -BHC の減少率は 80-90% を示した。菌体重と γ -BHC 減少率は、菌体 10-200 mg の実験範囲内では、菌体重の増加とともに減少率も増加した。これは死菌体を用いてもよく似た結果を得た。

これらの結果を総合すると培地に加えた γ -BHC の一部は菌体に吸収（あるいは吸着）されるものと推定される。なおこの菌体のまさい物中からも γ -BHC を検出し得たが、生菌体内に吸収された γ -BHC のその後の代謝については検討中である。