Effect of Insecticides on the Nodulation activity of *Rhizobium japonicum*

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殺虫劑가 根瘤菌의 根瘤形成에 미치는 영향

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ABSTRACT

The insecticides, Dithane-M45 and copper sulfate, were introduced in this experiments to elucidate their effects on the nodulation of soybean plant(*Glycine max* Meer) by *Rhizobium japonicum*. The nodulation activity was inhibited in accordance with increase of their concentration.

INTRODUCTION

Recently the pathological phenomena of the leguminous plants, such as bacterial pustle, bacterial blight, downy mildew, and brown spot etc., are getting to close the serious problems in agriculture. There have been many efforts to eliminate the pathogens or vectors, so as to spray the chemical insecticide so often to the leguminous plant. Frequent sprays of such chemicals were eventually resulted in pollution problems in both of plant and soil environment. The residue of insecticides in soil is believed to show some effects on the beneficial soil microbes as well as plant body. The author was interested in studying the relationships between the residues and nodulation activity of *Rhizobium japonicum*.

MATERIALS AND METHODS

1) Seedlings; the seeds of *Glycine max* Meer were sterilized with 0.5% $HgCl_2$ and rinsed with distilled water. To grow the seeds, fine sand was steri ized with steam heat for 2 days intermittent'y. After then, the seeds were incubated in the sand and p'aced in green house.

2) Preculture of *Rhizobium japonicum*; the nodule bacteria which were donated from Korean Association of Forest and Forest Products have been cultured in the Ashyby's mannto! agar (1953) for 2 days at 25°C.

And then the cultures were diluted with sterilized phosphate buffer to prepare the inoculum.

3) Culture medium for Plant; culture medium for sand culture was prepared on the basis of Knop's solution, modified as the nitrogen-free state. The composition was consisted of the followings; KH_2PO_4 0.25g, KCl 0.12g, $MgSO_4 \cdot 7H_2O$ 0,25g, $FeCl_3$ trace, $MnSO_4$ 7.5mg, $CaCO_3$ 1.0g and sterilized with 1 liter of tap water.

of nodulation by copper sulfate, which were increased from the concentration over standard dose. But there is no significance to be recognized among all treatments.

Fig. 1 and 2 were drawn by the data of Table 1 and 2 as the histogram. Referring to the figures, inhibition of nodulation by insecticides was decreased according to the time lapse. In treatment of copper sulfate, the inhibition of nodulation was also decreased, but the number of nodulation in control plants was higher than those in treatment at the end of the experiment.

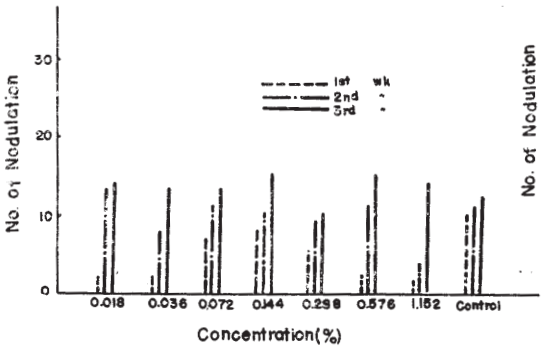

Fig. 1. Dithane-M45 treatment.

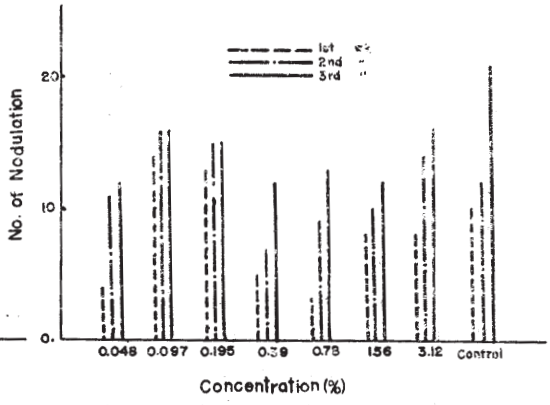

Fig. 2. Copper Sulfate treatmen+.

摘 要

메주콩(*Glycine max* Meer)에 근류균(*Rhizobium japonicum*)을 접종하고 2종의 insecticide, 즉 Dithane-M45와 Copper Sulfate를 처리해준 결과 다음과 같은 실험 성적을 얻었다.

Dithane-M45는 토양중에 잔류하여 농도가 증가함에 따라 근류균의 근류형성을 억제하는 것으로 확인되었으며 Copper sulfate는 통계처리 결과 상관계수 r의 값이 0.270으로 r〈0.5이므로 저해효과의 유의성을 찾을 수 없었다.

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4) Dilution series of Insecticide; Dithane-M45 (zinc-manganes ethylene-bisdithiocarbamate) and basic copper sulfate were all water soluble. Dithan-M45 is usually used as dilution of 0.14%. This dilution was made for standard concentration of treatment and the others were prepared in order of 0.018, 0.036, 0.072, 0.144, 0.288, 0.576, 1.152%, respectively. On the other hand, basic copper sulfate is usually used as dilution of 0.78%. Dilution series were prepared on the basis of the above concentration as the following orders; 0.048, 0.097, 0.195, 0.39, 0.78, 1.56, and 3.12%, respectively.

5) Inoculation of *Rhizobium japonicum;* five of sterilized seeds were sowed into pot and inoculated with diluted cultures of *Rhizobium japonicum* as about 10ml, which are equivalent to 5.15×10^6 microbes per milli liter. After then, buried with sand, the insecticides were sprayed into soil about 10 ml, respectively.

RESULTS AND DISCUSSION

Table 1 and 2 meant the number of nodulation at the root hair of soybean plants. It is generally accepted that the *Rhizobium japonicum* can be inoculated to form nodules only to the plant *Glycine* sp. (Vincent 1968, Fred 1932). Referring to the table 1 and 2, two kinds of insecticide showed its inhibition effects of nodulation on the second week after treatment(Russell 1966). In case of Dithane-M45 treatment, inhibition effects were getting to increase according to the increase of its concentration.

Braithwaite(1958) had already reported the inhibition of nodulation by insecticides, such as aldrin, dieldrin, chlordane, DDT, and BHC, and he asserted the effects of DDT and BHC on nodulation as the nodulation of plant were one fourth of the control plants. However, in this experiment, the plant which were treated with the concentration of 1.152% showed the number of nodulation as the results of above report.

Table 1. Nodulation number of *Glycine max* Meer by *Rhizobium japonicum.*

Week	1st	2nd	3rd	4th	total
DM%					
0.018	—	2	13	14	29
0.036	—	2	8	13	23
0.072	—	7	11	13	31
0.144	—	8	10	15	33
0.288	—	5	9	10	24
0.576	—	2	11	15	28
0.152	—	1	3	14	18
Control	—	10	11	12	33

☆ DM : Dithane-M45

Table 2. Nodulation number of *Glycine max* Meer by *Rhizobium japonicum.*

Week	1st	2nd	3rd	4th	Total
C.S%					
0.048	—	4	11	12	27
0.097	—	14	16	16	46
0.195	—	13	15	15	43
0.39	—	5	7	12	24
0.78	—	3	9	13	25
1.56	—	8	10	12	30
3.12	—	8	14	16	38
Control	—	10	12	21	43

☆ CS : Copper sulfate

The concentration of 1.152% is equivalent to 8 times of standard dose. Cn the other hand, Russell(1966) reported that significant inhibition had occurred to the plant when the concentration of insecticide is 3 times higher than standard dose. But the results of Russell's report is not comparable with this experiment.

Table 2 represents the inhibition effects