

**BACTERIAL NODULATION AND RELATIVE VIGOUR
OF VICIA FABA IN RESPONSE TO BACILLUS
MEGATERIUM STRAINS STIMULATED IN SOIL
TREATED WITH THE INSECTICIDE SEVIN
(N-methyl-1-naphthyl carbamate)**

By

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INTRODUCTION

An aspect of the indirect effect of the insecticide sevin (N-methyl-1-naphthyl carbamate) on soil micro-organisms is the inhibition of *Azotobacter* activity by bacteria stimulated in the soil treated with this insecticide (the authors, 1969 b). Of these bacteria, strains of *Bacillus megaterium* (2, 9 & 11) were the most resistant to sevin; strain 2, was able to utilize sevin as only carbon and nitrogen source (the authors, 1969 a). Bacterial nodulation of legumes is taken as criterion of the activity of *Rhizobium* spp. on their root system. This nodulation is supposed to exert developmental effects on the legume (Borodulina, 1962; Dorosinskii et al., 1963; Krasibukov and Melkumova, 1963; Bisset, 1963). Therefore, the formation of bacterial nodules and the relative vigour of the legume might be taken as criteria for the activity of *Rhizobium* under controlled conditions. Increase in *Rhizobium* activity might indicate an increase in the fertility level of the soil, and vice-versa. Since some bacteria stimulated in soil treated with the insecticide sevin have inhibited *Azotobacter* activity as mentioned above, it becomes important to elucidate the nature of effect of these bacteria on the activity of *Rhizobium*. This is the aim of the present investigation.

MATERIAL AND METHODS

Inoculating soil with bacteria :

Bacteria used in this investigation were strains 2, 9, and 11 of *B. megaterium*, previously found to be stimulated in sevin-treated soil (the authors 1969 a).

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They were grown in Czapek's liquid medium for 48 hours at 30° C. Suspensions of bacterial cells washed for 3 consecutive times in physiological saline were prepared. 5 ml. of a suspension served as an inoculum to 100 gm. soil. The inoculum was dropped on the surface of the soil which was spread in wide container. After 1 hour the soil was homogenized using a sterile soil spatula. The inoculated soils were dispensed in pots of 4 kg. capacity.

Setting up the experiment :

Vicia Faba was the legume used. The soil was a 2 mm. sieved garden soil. One of the soil treatments was application of sevin at a dose of 500 ug/gm. soil. Two of the investigated strains (9 & 11) were found stimulated at this dose. This treatment was made to compare its effect on nodulation and plant vigour with that of the investigated bacterial strains in the absence of sevin (their stimulator). Sevin was applied by adding the calculated amount to the soil which was then thoroughly homogenized.

Soil treatments were :-

- (a) Soil (control).
- (b) Soil treated with sevin (500 ug/gm).
- (c) Soils inoculated with bacteria.

Soaked seeds were put into the soil of the above treatments at a depth of about 2 cm. Not less than 20 plants were raised in every treatment. The soils were irrigated daily with equal volumes of water. Duration of the experiment was 5 weeks from sowing, when plants were excavated gently from the soil, the roots washed with water and the required determinations made.

RESULTS

It seemed that *B. megaterium* strains 2 and 9 have depressed the potentiality of flowering of *Vicia Faba* (Table 1). A profound influence has been observed being manifested by developing of branches in the axils of prophylls, a character observed in only 5% of control plants, as compared to about 4 times this value in experimental plants growing in soil inoculated with bacteria. In soil treated with " sevin ", the majority of the plants (75%) showed branching in the axils of their prophylls. In only one case (*B. megaterium*, strain 9) simple leaves have been recorded (Fig. 1).

The most important finding was that nodulation in all treatments was significantly inhibited (Table 2). This inhibition had been reflected on grown plants by developmental depressive action in soil treated with " sevin " manifested by significant decreases of root length and fresh and dry weights. Some of these harmful effects were the relative thickening and shortness of the primary

Table 1 Some developmental criteria of *Vicia Faba* in response to the effect of "Sevin" or bacteria stimulated in "Sevin" treated soil.

Control and treatments (Soil with)	Flowering %	% of plants with branching in the axils of prophylls	% of plants hav- ing simple leaves
No treatment (control)	15.0	5.0	0.0
Sevin (500 ug/gm)	15.0	75.0	0.0
B. megaterium, 2	0.0	20.0	0.0
B. megaterium, 9	5.0	20.0	5.0
B. megaterium 11	20.0	20.0	0.0

Table 2 : Effect of sevin and bacteria stimulated in soil treated with it on the relative vigour of *Vicia Faba*. (+), significant increase, and (-) Significant decrease.

Criteria	Control soil	Soil treated with			
		Sevin (500 ug/gm)	B. megaterium		
			Strain 2	Strain 9	Strain 11
<i>Shoot :</i>					
Number of Leaves/plant	7.0	8.6	7.9	7.4	7.8
Length (cm)	11.0	14.5	17.4(+)	16.3	18.4(+)
Fresh wt. (gm)	4.1	4.6	4.5	3.7	4.2
Oven dry wt. (mg)	499.8	402.5	338.5	379.8(-)	318.4(-)
<i>Root :</i>					
Length (cm)	16.8	5.1(-)	15.5	18.9	17.6
Adventitious (No/plant)	0.4	3.5	3.4(+)	6.0 (+)	3.1(+)
Nodules (No/plant)	12.9	1.2(-)	1.7(-)	0.05(-)	1.25(-)
Fresh wt. (gm)	5.1	2.2(-)	4.4	2.6(-)	4.6
Oven dry wt (mg)	383.0	210.2(-)	308.1	275.1	304.4

root. There were significant increases of numbers of adventitious roots of plants growing in soil treated with bacteria.

The dry matter of the shoot was significantly effected in soils inoculated with *B. megaterium* strains 9 & 11 : one of these (*B. megaterium* strain 9) had shown a depressive effect on potentiality of flowering.



Fig. 1 — Simple leaves of *Vicia Faba* recorded in some plants raised in soil inoculated with *B. megaterium*, 9.

DISCUSSION

The most important finding was the significant deleterious effect of sevin or the investigated strains of *B. megaterium*, on bacterial nodule formation in *Vicia Faba*. This means that sevin and its stimulated bacteria have exerted a depressive effect on the activity of *Rhizobium* spp. in soil. This finding together with the previously recorded inhibitory effect on *Azotobacter* activity (Elwan and Khodair, 1969 b) in soil, might indicate the necessity of taking great caution when using this insecticide.

Depressing the activity of both symbiotic and nonsymbiotic nitrogen fixers would deteriorate the fertility standards of the soil.

In soil treated with sevin, the inhibition of nodules was significantly reflected on some root system criteria causing decrease in the length of the primary root, and the fresh and oven dry weights of the whole root. However, it seemed that the investigated bacteria had supplied the plant with certain growth factors which induced significantly the adventitious root formation. This induction seemed to compensate for the harmful effect of the nodule-inhibiting factors, at least in case of *B. megaterium* strains 2 & 11, where no significant depressive action was reported on either the shoot or the root systems. On the contrary, the length of the shoot system was significantly increased.

The increase of branching in the axils of prophylls in sevin treated soil more than the other treatments (soils inoculated with bacteria) might give indication of some other bacterial stimulation in the soil exerting this recorded effect.

As for the finding of the previous investigators, the antagonistic effect of microorganisms on *Rhizobium* was recorded (e.g. Robison, 1946; Thornton *et al.*, 1950; Hattingh and Louw, 1966). Campillo (1947) found that some aerobic sporulating bacteria possess a marked antagonistic reaction toward *Rhizobium*, seriously interfering with nodule formation of leguminous plants. On the other hand, Krasilnikov *et al.* (1944) recorded some bacteria which stimulate the growth and propagation of nodule bacteria. Anderson (1957) concluded that the effect of an organism on the plant-Rhizobia association seems to depend on the species of the organism and on the strain of Rhizobia used in the tests. Most of the organisms which caused poorer plant growth seemed to affect the nodulation. He suggested that the organism and Rhizobia in the presence of a legume compete with each other for essential nutrients. In this investigation, however, some strains (2 & 11) induced the development of some plant characters such as length of the primary root and adventitious root formation, at the time when nodulation was seriously affected. Therefore, it seems that the factors responsible for decreasing nodulation are functional bacterial exudates rather than being competition for essential nutrients.

With regard to the effect of the insecticides on symbiotic nitrogen fixing bacteria, many authors have found that normal field application of insecticides had no inhibitory effect (Appleman and Sears, 1946 ; Fufts and Payne, 1947 ; Wilson & Choudri, 1946 ; Sinkover and Shenefelt, 1951). On the other hand, higher concentrations exerted harmful effect (Wilson and Choudri, 1948 ; Sanchez, 1954 ; Abou El-Fadl & Fahmy, 1958). However, other insecticides stimulated the symbiotic nitrogen fixing bacteria (Pederson, 1949 ; Dudnik, 1956 ; Lakocy, 1957 ; Abou El-Fadl & Fahmy, 1958 ; Hamed 1965).

ACKNOWLEDGEMENT

The authors offer their thanks to Professors A.H. Montasir and M.G.A. Hafez for encouragement.

SUMMARY

Bacillus megaterium strains 2, 9 & 11 were previously found stimulated in soil treated with the insecticide sevin (N - methyl - 1 - naphthyl carbamate). They were also previously found to inhibit the activity of *Azotobacter* in the soil. The present investigation is an attempt to elucidate their effect on the activity of *Rhizobium* spp. in the soil as manifested by the bacterial nodulation and relative vigour of *Vicia Faba* (broad bean). Each of the bacterial strains was inoculated into the soil in which viable broad bean seeds were allowed to develop for 5 weeks. A soil was also treated with sevin (500 $\mu\text{g}/\text{gm}$) for the sake of comparison. Controls were untreated soils.

The most important finding was that application of sevin to soil and its inoculation with the bacterial strains have significantly inhibited bacterial nodulation. In case of sevin treated soil, this inhibition was reflected on some root system criteria causing decrease in the length & fresh and dry weights. In case of bacteria the inhibition was not similarly reflected on the root system. This was correlated with the significant induction of adventitious roots by the bacterial strains, a character not observed in sevin - treated soil.

Results indicated an inhibitory effect on the ability of *Rhizobium* to nodulate the roots of broad bean.

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