

EFFECT OF THE INSECTICIDE SEVIN  
( N-methyl-1-naphthyl carbamate )  
ON SOME SOIL MICROORGANISMS

By

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INTRODUCTION

The increasing use of the insecticides to control pests directed the attention of many investigators towards elucidating their effects on various aspects of life in the soil. Investigations carried out on the effect of insecticides on soil microorganisms dealt for the most part with chlorinated hydrocarbons (e.g. Wilson and Choudri, 1946; Smith and Wenzel, 1948; Fletcher and Bollen, 1954; Jonson and Falreus, 1960; Hamed, 1965; Ishizawa and Matsuguchi, 1966; Altman and Lawlor, 1966; El-Mokadem, 1968). Few investigations were made on the carbamate insecticides (El-Hoseiny 1964; Taha *et al.*, 1966). Therefore, it was desirable to investigate the effect of sevin (N-methyl-1-naphthyl carbamate) on the soil microorganisms using a wide range of doses, applied for two consecutive times, under controlled conditions, along a relatively long time (about 4 months). The aim was to reveal the duration of the effects on the microbial groups which might be reflected on the soil biological potency. This paper represents a part of investigations on the effect of chemicals and drugs on the soil microorganisms aiming at revealing some microbial stimulations which might exert effects in the soil as regards its potentiality of harbouring pathogens or its biotic vigour as manifested by the development of higher plants or beneficial microbes.

MATERIAL AND METHODS

*Experimental soil* : This was a garden soil receiving annual supply of compost. It was collected from the horizon of 2-5 in depth, air dried, sieved in a 2 mm sieve and stored in wide mouth dark bottles.

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*Sevin Application* : Since condensation of sevin may take place in the soil, the effect of various doses was attempted. Doses of 0.5, 2.5, 5, 10, 50, 100, and 500  $\mu\text{g}/\text{gm}$ . soil were applied. Any dose of these may be represented in the soil at one time or another.

For applying the insecticide, 100 gm. of the experimental soil were taken in a weighed sterile conical flask of 250 ml. capacity. The required weight of sevin powder was added to soil and stirred to homogenize using a sterile spatula. In applying lower doses, 1 gm. of sevin was diluted several times by known weight of experimental soil and calculated weight was added to soil as required. Second application of sevin was made after 6 weeks from the start.

*Counting of total viable bacteria, viable bacterial spores, and actinomycetes* : Soil extract agar (pH 7) supplemented with 0.125% sucrose and 0.025%  $\text{KH}_2\text{PO}_4$  was used for counts. Decimal plate culture technique was followed. Inocula for spore counts were heated at 90 C for 10 mins. in 5 ml. aliquots of the suitable dilutions. All counts were calculated per 1 gm. soil.

*Counting of Azotobacter* : The technique of Elwan and El Sayed, 1964, has been used in which a load of a standardized sterile spatula (16 mg. of the present soil) was sprayed on a nitrogen-free agar plate.

*Counting of acid producing bacteria* : 900 ml. of Bunt and Rovira (1955) medium as devised by Louw and Webley (1959) were mixed before sterilization, with 100 ml. of 1.5% (w/v) of  $\text{CaCO}_3$  in 0.5% (w/v) gum arabic. Bacterial colonies producing halos on plates were considered as acid-producing.

*Counting of fungi* : The soil plate culture technique using Czapek-yeast extract agar (Warecup, 1950) was followed using a load of the above mentioned standardized spatula from aseptically air dried soil sample. Identification of fungi was made using the keys of Gilman (1962).

*Incubation* : All counting plates were incubated at 29-31 C. Time suitable for incubation was found to be 3 days for *Azotobacter*, 5 days for total viable bacteria and acid producers, 6 days for fungi and 7 days for viable spores.

*Expression of results* : The rhythm of effect of various doses is indicated by plotting treated: control ratios in graphs.

## RESULTS

*Total viable bacteria* : Application of the insecticide sevin did not apparently seem to exert great effect on the total viable bacteria. In some cases slight stimulatory, in others slight inhibitory effects were recorded (Fig. 1). For instance, in the first treatment, total viable bacteria seemed to be stimulated one week and after from start at the dose of 100,  $\mu\text{g}/\text{gm}$  soil. This stimulatory response was

nearly the highest one recorded. However, on applying sevin for a second time, the observed stimulation rapidly disappeared in 2 days, and a stimulation followed along 3 weeks whereafter a relatively slight inhibition took place for some weeks more ( Fig. 1). The common stimulatory feature which covered the first application period was not repeated again. However, it can be said that the second application of sevin depleted total viable bacteria after two days, irrespective to the dose applied.

*Viable bacterial spores:* With the exception of few determinations, the common feature was the favouring of sporulation irrespective to the dose level ( Fig. 1 )

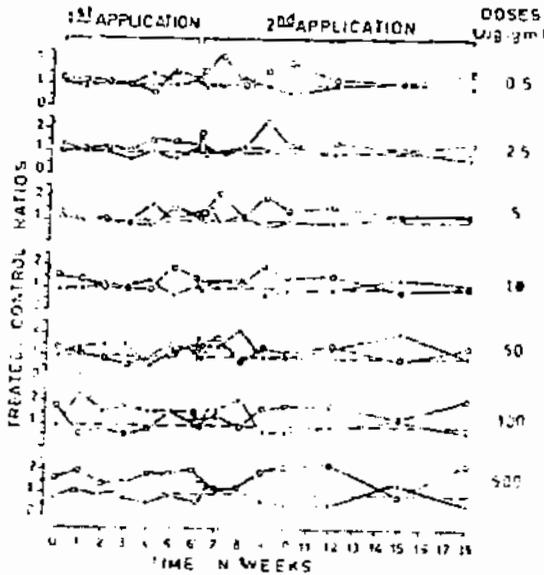


Fig. 1 — Responses of the total viable bacteria (dots) and the viable bacterial spores (circles), to the application of sevin to the soil.

*Actinomycetes :* Fluctuations ( Fig. 2 ) could be observed in the records of any dose after both first and second applications. However, these fluctuations were relatively more drastic at higher doses than at lower ones.

*Acid producing bacteria :* In most cases, acid producers were higher in treatment values than in control ones, directly after application ( Fig. 3 ). The effect after 2 weeks of the first application was not similar to that of the second application. It might be said in general that there was a tendency towards stimulation effect some weeks after the second application in most of the doses. However, fluctuations occurred in every dose. A decreasing tendency was observed, 4 weeks and after in the second application ( 10 weeks from start, Fig. 3 ). This continued

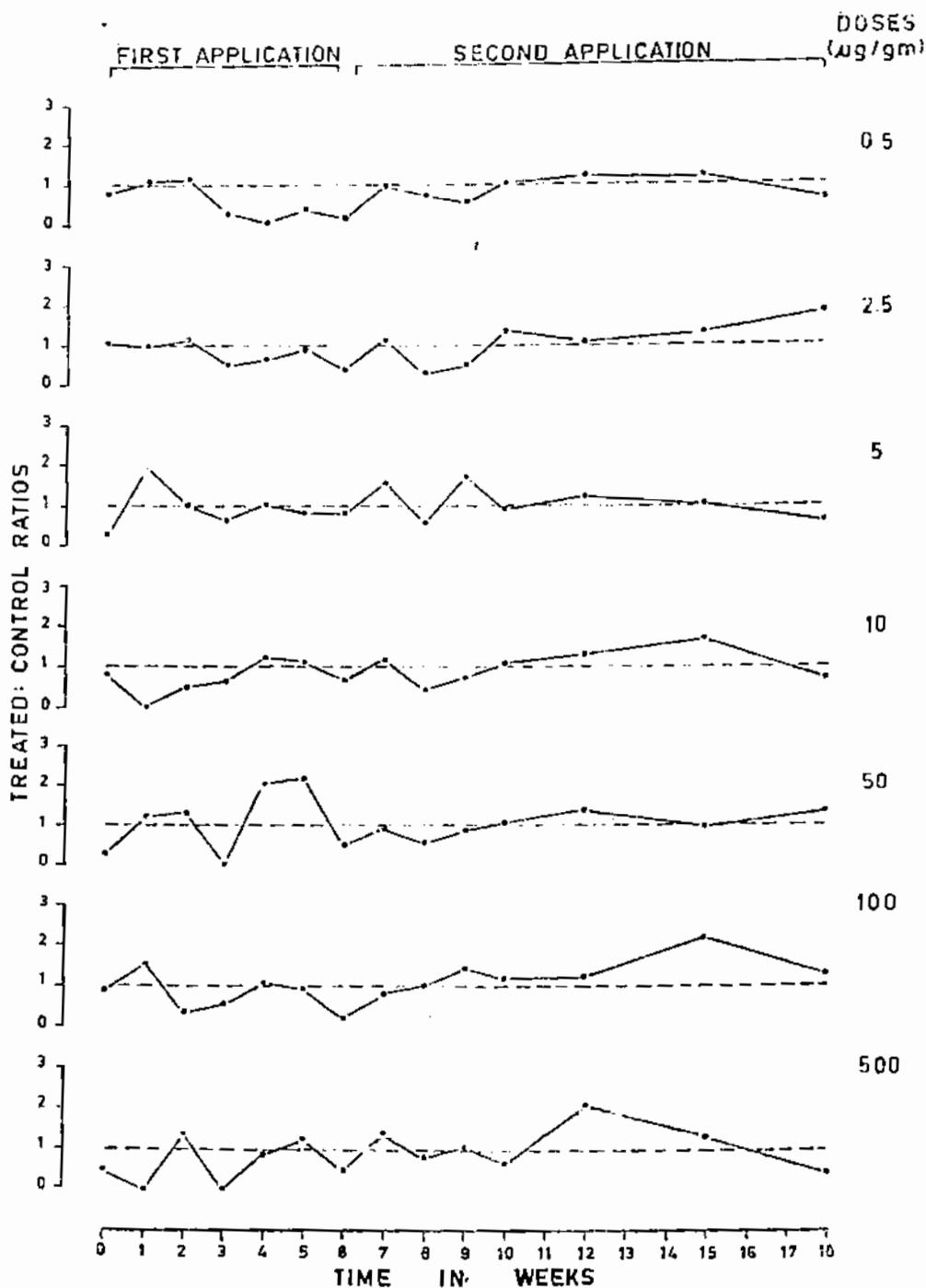


Fig. 2 — Responses of actinomycetes to the application of sevin to the soil.

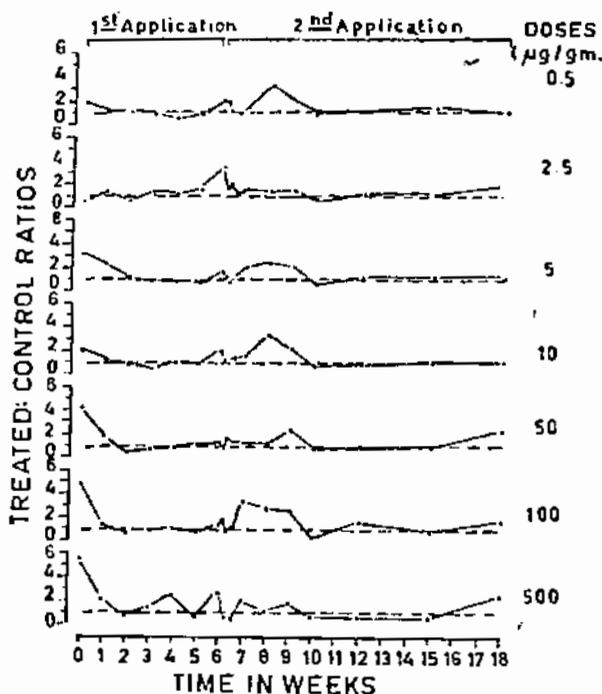


Fig. 3 — Responses of the total acid producing bacetria, to the application of sevin to the soil.

for about 5 weeks at the doses of 50 ug/gm, 100 ug/gm, and 500 ug/gm with the exception of one count at the dose of 100 ug/gm.

*Azotobacter* : *Azotobacter* was seriously affected at higher doses of sevin. This becomes more evident at the dose of 500 ug/gm when treated :control ratio decreased markedly below unity as compared with lower doses. The depressive effect exerted on *Azotobacter* at such dose lasted for 18 weeks covering the 2 consecutive applications with the exception of one count only ( Fig. 4 ).

*Fungi* : A common feature of the total numbers of fungi was the increase of the depression effect with the increase of the dose (Fig. 5) along about 12 weeks from the start.

*Aspergillus niger* represented the major existence among the fungal species on counting plates being about 50% of the total fungi. *Aspergilli*, in general were the most dominant species. *A. niger*, however, responded to sevin application in a manner different from that of the total fungi. It was not depressed at higher doses as at lower ones ( Fig. 5 ).

*A. flavus* : ( control distribution frequency of 3 - 17% of the total fungi) was highly stimulated in all doses of sevin ( Fig. 5 ) ; sometimes, no single depressive

effect was recorded (doses of 2.5 and 5  $\mu\text{g/gm}$ ). A maximum treated : control value of about 10 was recorded at the dose of 10  $\mu\text{g/gm}$  soil. An evident observation was that maximal stimulation effect of this fungus occurred 1 week after the first and 2 weeks after the second applications.

*A. terreus* (control distribution frequency of 2.5 - 14% of the total fungi) was similar to total fungi in response to sevin application.

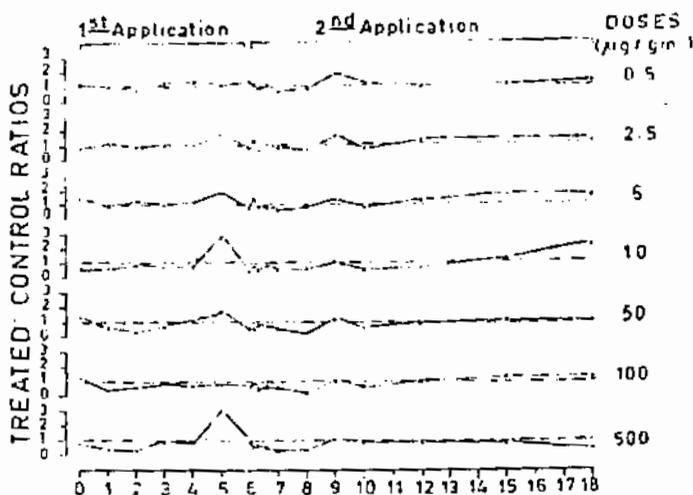


Fig. 4 — Responses of *Azotobacter* to the application of sevin to the soil.

*A. sydowii* and *A. nidulans* (of moderate frequencies in control soil) were more or less similar to *A. terreus* in their responses to sevin; both the higher doses and application for a second time, tended to depress them.

*Penicillii*, *Fusaria*, *Helminthosporium* sp. and other fungi were all of low frequency and were not of regular appearance on control plates at the same dilution level. However, they were not recorded on concentration more than 5  $\mu\text{g/gm}$  with few exceptions.

## DISCUSSION

The observed stimulations which followed in many cases inhibitory effects might indicate an attempt of some groups of bacteria to flourish when inhibitory action was exerted on other groups. This may interpret the common fluctuations and variability of effect on various groups or species of Microorganisms. The soil - even under controlled conditions - is never a stationary system. Living entities in the soil are dynamically equilibrated. Characteristics of the biodynamics of a soil will specify the biotic potency of this soil. It is difficult to specify

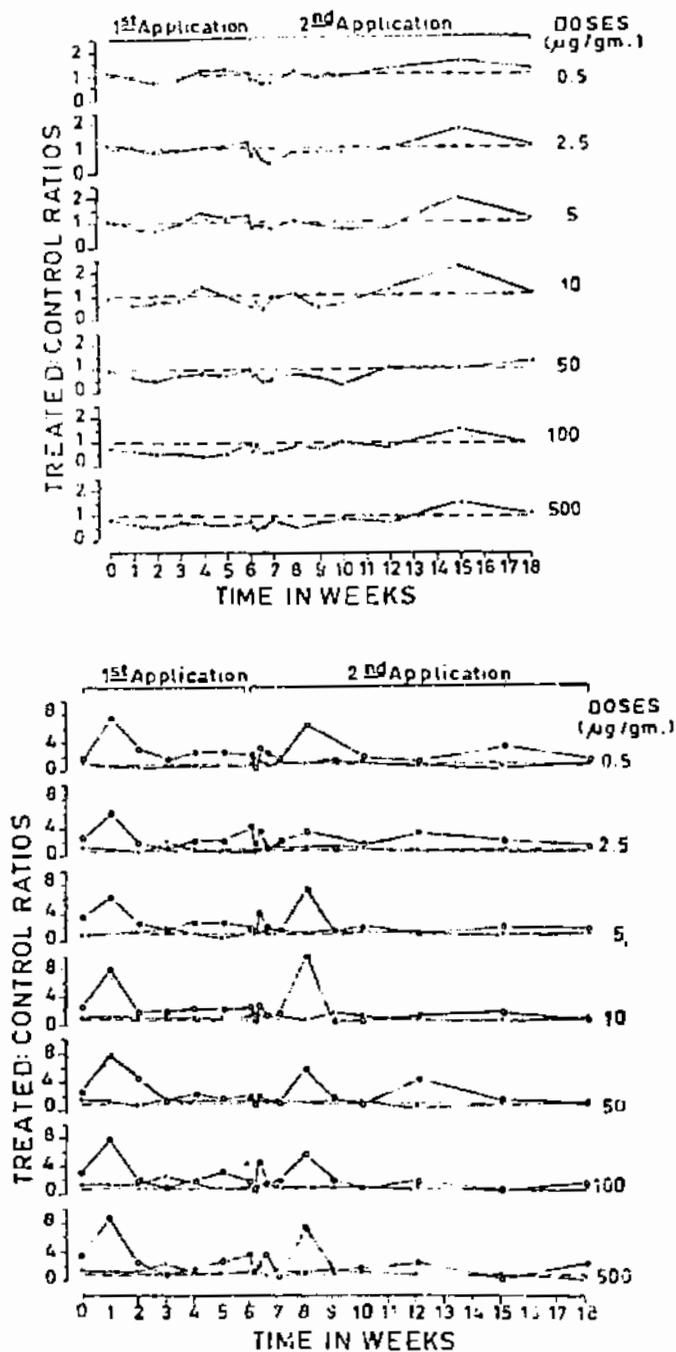


Fig. 5 — Responses of the total fungi upper and 2 of their dominant species lower : (*Aspergillus niger* indicated by dots and *A. flavus* by circles) to the application of sevin to the soil.

the nature of the evolving group in the dynamic interaction of microbes in the soil. A quick tool is required to follow various stimulatory or inhibitory effects on bacteria. Morphologically distinct bacterial colonies developing on counting plates are felt helpful in this respect. This will be the subject of another investigation.

Although results have shown that responses of microbes were for the most part fluctuating, there were some effects that extended for long time such as the inhibition of the total mycoflora.

The differences in responses to application of sevin between total determinations and their species (such as fungi) or group components (such as bacteria) are against fixing theories claiming that fluctuations in numbers of microorganisms are due to the activity of only one group (protozoa) of these microorganisms (Ruessel and Hatchinson, 1913 protozoan theory of microbial equilibrium in soil, as quoted by Thimann, 1967). Several groups or species are responsible for the observed fluctuations. Many authors have reported that organic pesticides may influence the activities of soil microbes by serving as carbon and energy source for the selective growth and multiplication of specific organisms (e.g. Zo Bell 1946; Walker and Newman, 1956; Eno & Everett, 1958; Martin & Pratt, 1958; Martin, 1963; Altman and Lawlor, 1966).

Comparing the effects of sevin on total viable bacteria with those of certain other insecticides, some differences are obtained. Total viable bacteria were very little if at all affected by aldrin or benzene hexachloride (Wilson & Choudri 1948; Jounson & Fahreus, 1960) and were stimulated by chlorinated camphene, pentachlorophenol and lindane (Smith and Wenzel, 1948; Ishizawa and Matsuguchi, 1966; El-Mokadem 1968). However the effect of sevin was more or less similar to the effect of dieldrin (El-Mokadem, 1968) who observed an early stimulation followed by an inhibition and then a marked increase in some cases. In the pretreated soil, marked inhibition was noticed which was more pronounced in higher dose. El-hoseiny, 1964 observed slight deleterious effects of sevin early in the experimental period of a certain dose (2.5 Kg/feddan). He observed higher counts later in the experimental period. This was claimed to be due to the growth of the mutants and adapted organisms on the dead microbial protein of the susceptible organisms. Results of the present investigation are not in parallel with the previous author's claim. If the stimulation observed was due to the flourishing of adapted organisms the second application of the insecticide at the same dose would have increased this stimulatory effect which was not the case in many cases. Two days after second application, the total viable bacteria were depleted irrespective to the dose applied. It must be

mentioned that the duration of the experiment in the previous investigation was only 60 days at a maximum dose of 2.5 kg feddan ( 2.5 ug gm soil, considering 4000 m<sup>2</sup> with 20 cm depth as 1000 tons ) whereas the present investigation was on 7 doses covering a range of 0.5—500 ug/gm. Variability of temperature and water content, and pollution from the air microflora were eliminated. It seems more likely that the activity of the *evolving* microbes through exuding certain, stimulators or inhibitors, was the factor responsible for the observed effects.

Favouring of bacterial sporulation was similar to the effect of lindane ( El-Mokadem, 1968 ), and not similar to the results of El-hoseiny, 1961 who found that spore counts showed marked reduction later after application of sevin and other insecticides. Sporulation increase might be reflected on the general activity of bacteria in the soil. However, actively growing vegetative bacterial cells represent more than 90% of the total bacteria in nearly all the doses. This might show that the general viability of bacteria in the soil was not seriously injured by sevin application, although sporulation was generally induced.

The observed fluctuating response of actinomycetes was different from the response to insecticides such as benzene hexachloride ( Hauke, 1958 ), endrin ( Hamed, 1965 ) where no effect was observed, or lindane ( El - Mokadem, 1968 ) where stimulation occurred. El-hoseiny, (1961) observed no deleterious effect of sevin on actinomycetes.

Results showed indication of an indirect effect on acid producing bacteria. Both depressive and stimulative responses were recorded, with no parallelism with either the total bacteria or any of the other groups, irrespective to dose application. Any one of such responses may last for a time as long as several weeks ; this occurred at the dose levels of 50 and 500 ug/gm soil where a decreasing tendency lasted for 5 weeks. This might exert important effects on soil biological potency.

The depressive effect on *Azotobacter* few days after second application might show a direct effect. However, indirect effect in an ever changing system could not be excluded. In evidence of this was the occasional recorded tendencies of stimulating *Azotobacter*. The depression of *Azotobacter* for a relatively long time at the dose of 500 ug, might notify the significance of controlling dose application of this insecticide. Inhibition of *Azotobacter* in soil was similarly observed in soil treated with DDT ( Callao and Montoya, 1956 ) and endrin ( Hamed, 1965 ). El-hoseiny, 1961 observed inhibition of *Azotobacter* by "sevin" at the doses of 1.2 and double field application.

Results showed that the depression effect on the total fungi seemed to be direct, since it was observed in all doses after one week of both first and second

applications. The observed dominance of aspergilli might help in the decomposition processes of the organic matter complexes rendering them accessible for utilization by both microorganisms and higher plants

The observed response of *A. flavus* favours the necessity of investigating physiological groups (individual species if possible) of microbes rather than their totals, if the action of a substance is required to be determined; the percentages of such groups or individual species in the total population have to be the criteria used for comparison (Elwan and Diab, 1969). Response of totals might mask an opposite response of their species or group components. This was evident in *A. flavus* response.

It can be concluded that although a depressive effect of total fungi was recorded yet a stimulatory effect of some species components was observed. The contribution of the stimulated fungal species to the physical and biological characteristics of the soil, needs evaluation with special reference to the relation of their activity and sporulation levels to the soil biological potency.

The response of total fungi to sevin was dissimilar to their response to other insecticides such as chlordane which increased the numbers of fungi (Smith and Wenzel, 1948).

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#### SUMMARY

Two consecutive applications of various doses (0.5, 2.5, 5, 10, 50, 100, 500  $\mu\text{g}/\text{gm}$  soil) of the insecticide sevin (N-methyl-1-naphthyl carbamate) did not exert great effect on the total viable bacteria, as determined by the decimal dilution plate culture technique on supplemented soil extract. In some cases, slight stimulatory and in others slight inhibitory effects were recorded. The consequential inhibition and stimulation for several times at various doses revealed a dynamic (and not static) microbial response, indicating an ever changing microbial balance and not a stationary one, in response to application.

Sevin application favoured bacterial sporulation, greatly or slightly, irrespective of the dose level. Fluctuations were observed in the records of counting actinomycetes as determined by the decimal dilution plate culture technique on supplemented soil extract. The same was observed with regard to the response of acid producing bacteria. *Azotobacter* as determined by the standardized spatula spray technique (air dry 16 mg of the present soil sprayed on N-free agar plate) was seriously affected at higher doses. The lasting of this effect for 4 months'

is suggestive of being direct. However, fluctuations at lower doses of second application, suggest an indirect effect through the action of microbes dominating at certain intervals along the experiment.

Total fungi as determined by a devised soil plate technique on Czapek's yeast extract were depressed, the depression increased with the dose increase. *Aspergillus niger* (the most dominant species) responded to sevin application in different from the response of the total fungi. This indicated that the response of (totals) might mask an opposite response of their species or group components. In evidence of this, was the stimulation of *A. flavus* at the time of inhibition of total fungi. Evaluation is required, of the importance of stimulated microbes in soil biological potency.

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