BEHAVIOR OF THE FUNGICIDE DEXON IN SOIL

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ABSTRACT

The behavior of fungicide Dexon (p-*dimethylaminobenzenediazo sodium sulfonate*) *in soil was studied. The fungicide disappeared from soil within* 100 *days. Dexon application slightly stimulated the total counts of bacteria, actinomycetes and fungi. The fungicide inhibited the conversion of nitrite to nitrate more intensely than that of ammonia to nitrite. At the same time nitrite oxidation by resting cells of* Nitrobacter winogradskyi *was not affected by this fungicide as revealed by manometric studies. In view of these observations the suggestion that Dexon affects mainly the heterotrophic nitrification in soil is made. N, N-Dimethyl-*p-*phenylene-diamine (DMPDA) which was earlier found to be the first breakdown product of Dexon was noted to be equally toxic to* Pythium, *the pathogen against which Dexon is mainly used.*

Key words: Dexon, soil fungicide, pesticide, *Pythium*, *Pseudomonas fragi*, N, N-dimethyl-*p*-phenylenediamine.

INTRODUCTION

Chemical treatment of seeds and soil for the control of plant pathogens while aiding in maximum yields of crops has also been recognized to cause unexpected damage to the environment [1]. We have now realized that detailed investigations on the fate and behavior of each pesticide must be conducted before recommending it for large scale agricultural use. Dexon (*p*-dimethylaminobenzenediazo sodium sulfonate) is a soil fungicide used to protect seedlings from ' damping-off ' and ' root rot ' diseases. Alconero and Hagedorn [2] have reported the effect of this fungicide on the soil mycoflora. We have earlier reported that Dexon is degraded to N, N-dimethyl-*p*-phenylenediamine (DMPDA) by a bacterial strain, *Pseudomonas fragi* Bk_9 obtained from soil [3]. However a detailed picture on the behavior of Dexon in soil is lacking. Therefore this study was undertaken and the results presented in this communication.

Material and Methods

Soil.—Red, sandy loam soil of Bangalore passed through 2 mm sieve was used in all the studies. The properties of the soil determined by the standard methods [4] are detailed below:

Type	*Red sandy loam*
sand	8·9 per cent
silt	4·8 per cent
clay	36·3 per cent
organic carbon	1·02 per cent
total nitrogen	0·08 to 0·1 per cent
cation exchange capacity	14–16 m. eq. /100 g
pH	7·0–7·4
water holding capacity (WHC)	33·3 ml/100 g.

Porcelain pots were filled with soil and Dexon (70 per cent wettable powder) was incorporated as a drench at 0, 100 and 200 ppm levels. Tap water was added to bring the moisture to approximately 60 per cent of the water holding capacity (WHC) of the soil and maintained at that level.

Enumeration of microbial population.—Soil samples in duplicate were collected from the top 3 cm and plated following serial dilution technique. Bacteria, actinomycetes and fungi were plated on soil extract agar [5], starch-casein hydrolysate agar [6] and rosebengal-streptomycin agar [7] media respectively. The plates were incubated for 3 to 7 days at 30° C after which colony counts were taken. In all the experiments duplicates were kept in each of the 2 dilutions plated.

Nitrification studies.—Air dry 2 mm sieved soil (400 g) was taken in 300 ml black coated polyethylene beakers. Dexon was incorporated at 3 levels, *i.e.*, 0, 1,000 and 2,000 ppm as a soil mix. Moisture level was maintained at 60 per cent WHC of the soil and it was corrected once in 2 days.

Determination of nitrite and nitrate in soil.—Soil (10 g) from the above beakers was drawn in duplicate at weekly intervals, nitrite and nitrate nitrogen in these samples were determined following the usual methods [8].

Manometric studies with Nitrobacter winogradskyi.—*Nitrobacter winogradskyi* obtained from Dr. S. W. Watson (Woods Hole Oceanographic

Institute, Woods Hole, Mass 02543) was checked for purity and was found to be free from heterotrophs. This organism was grown in mineral medium [9] in a 15 l pyrex glass jar. Sterile nitrite solution was periodically added till about 120 m moles/l of nitrite was oxidised, after which the cells were harvested and washed with 0·1 M Tris. HCl buffer (pH 7·8) and used in manometric studies [9, 10]. Oxygen uptake by these cells using $NaNO_2$ as the substrate along with various concentrations of Dexon was followed for 180 min.

Since Dexon is sensitive to light, experiments were carried out under dim yellow-green light [11].

Residual Dexon estimation.—Duplicate soil samples were collected from each Dexon treated pot at 3 different depths (0–10, 10–20 and 20–30 cm) using a soil auger. Dexon was then extracted in 1 per cent sodium sulfite, coupled with resorcinol for 30 min in alkaline condition under bright light, extracted in benzene and estimated colorimetrically following the method of Anderson and Adams [12].

Growth pattern of Pseudomonas fragi Bk_9.—Seubert's medium [13] containing 0·5 per cent glucose with or without Dexon in 500 ml conical flask was seeded with *Pseudomonas fragi* Bk_9 and incubated on a rotary shaker at 30° C in darkness. Growth was followed turbidimetrically using a Bausch and Lomb colorimeter (600 nm) at 60 min. intervals.

Formation and detection of DMPDA in soil.—Soil treated with Dexon from the above pots was extracted for 30 min with water. After centrifuging at 1,000 × *g* for 5 min the supernatant was taken and pH was adjusted to 8 with 1 N NaOH and extracted with an equal volume of ether. DMPDA in the ether layer was tested with Ehrlich's reagent [3].

Effect of Dexon and its metabolite DMPDA on the growth of Pythium aphanidermatum.—*P. aphanidermatum* (obtained from the Mycology Division, Indian Agricultural Research Institute, New Delhi) was used in this study. The extent of growth of *Pythium aphanidermatum* in the presence of Dexon or DMPDA was evaluated both in liquid as well as on solid media. The medium suggested by Hills and Leach [11] was prepared and distributed 100 ml amounts in 500 ml Erlenmeyer flasks. Filter sterilized Dexon or DMPDA was added to yield the required final concentrations. Whenever needed it was solidified with 2 per cent agar and poured into plates.

The flasks and the plates were inoculated with discs (3 mm, dia), from the margin of a young colony of *Pythium aphanidermatum*. All the plates

and flasks were incubated at 30° C. Studies were conducted in duplicate. The fungal growth on the plate was measured after 24 hr and the dry weight of the mycelial mat in the liquid culture was taken after 44 hr.

RESULTS

Effect of Dexon on soil microorganisms.—Soil microbial population was not adversely affected by the fungicide treatment as shown in Fig. 1. On the other hand there was an increase of 57 per cent in the fungal population after 15 days of treatment. Similarly bacteria showed about 60 per cent and actinomycetes 55 per cent increase in this period of time, though sudden spurt in the population during the first 15 days could be partly due to the moistening of air dry soil. However it is interesting to note that the higher levels are generally maintained throughout the period of study.

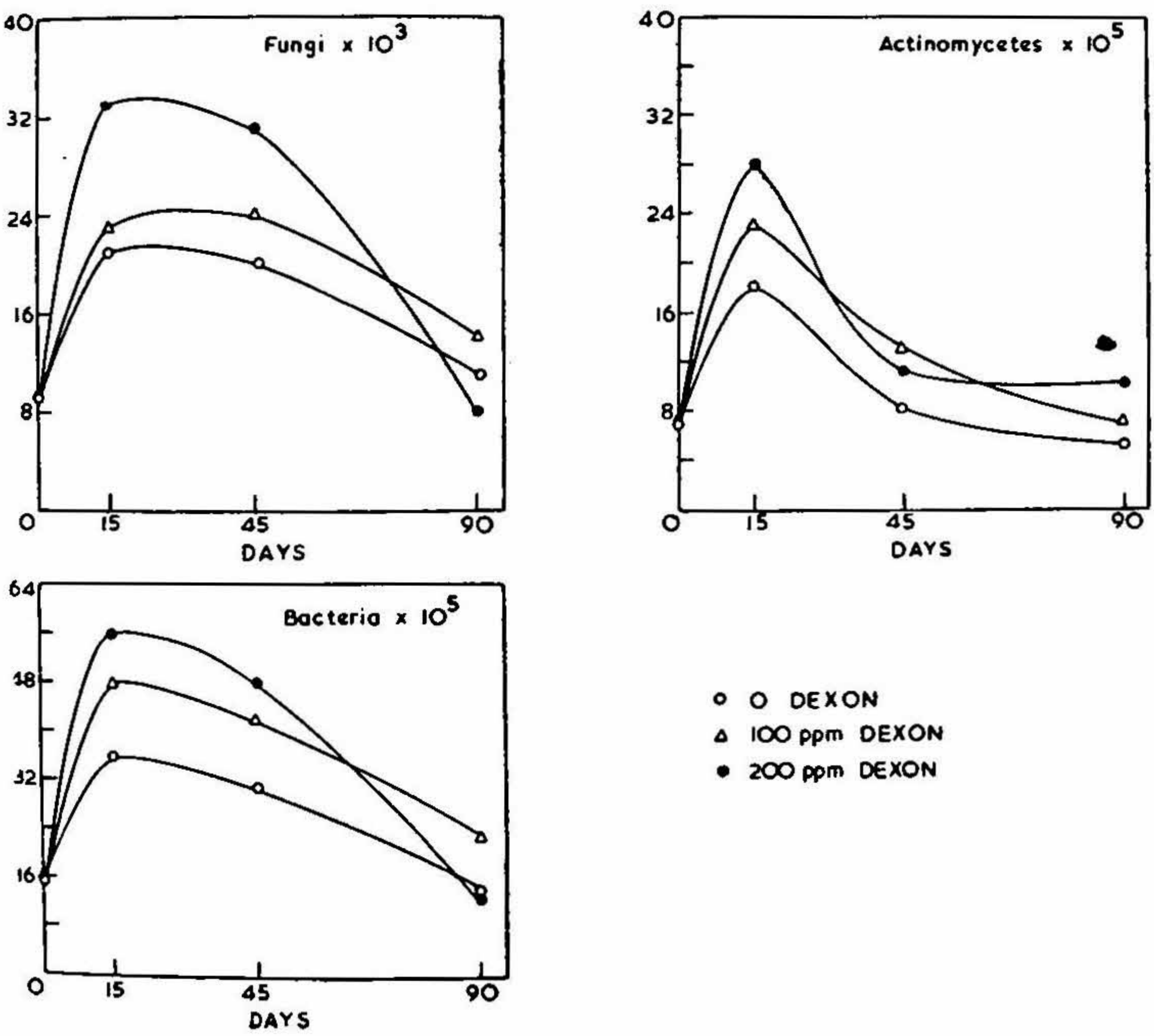

FIG. 1. Effect of Dexon on soil microbial population.

Effect on nitrification.—Effect of Dexon on nitrification is shown in Fig. 2. While nitrite formation is increased by Dexon treatment, nitrate production is retarded. The immediate temptation is to conclude that activities of *Nitrobacter* are susceptible to Dexon action, but that of *Nitrosomonas* are not. To further examine this phenomenon, oxygen uptake by resting cells of *Nitrobacter winogradskyi* was studied in a Warburg manometer. We observed that the fungicide had absolutely no effect on the process (Table I). This suggests that the inhibition of nitration by Dexon in soil might be by affecting the heterotrophic nitrifiers rather than the autotrophic nitrifiers. Of course the inhibition of nitrite oxidation observed could also be the result of the suppression of the multiplication of *Nitrobacter* in soil by Dexon.

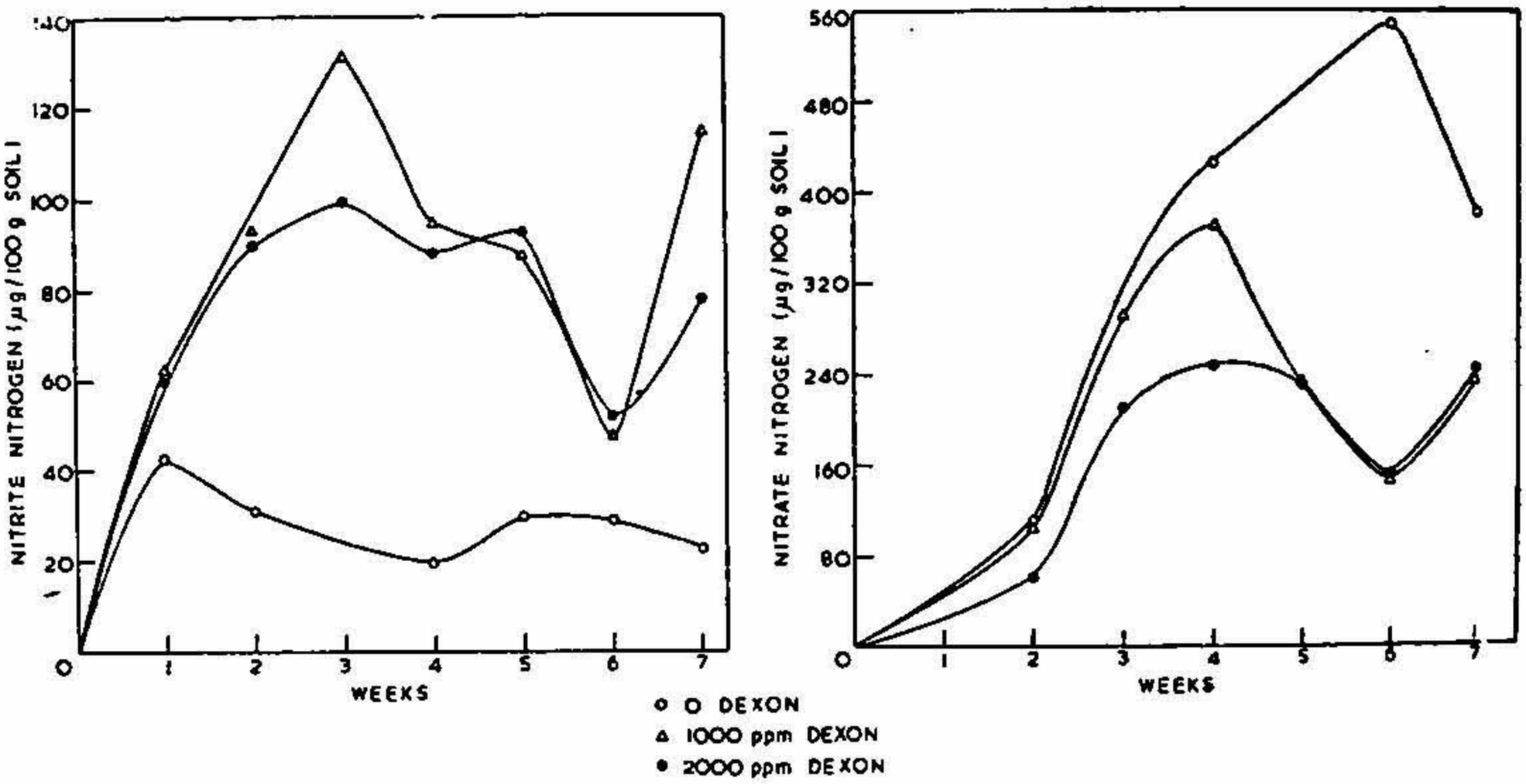

FIG. 2. Effect of Dexon on nitrification in soil.

Persistence of Dexon.—Dexon is found to be short lived in the soil. Residual level of Dexon at different depths at the end of 75 and 96 days after the treatment is given in Table II. It is clear that Dexon got distributed throughout the soil and slight accumulation occurred at the deeper regions. The fungicide disappeared from the soil rather fast and less than 1 per cent remained after 3 months.

Influence of Dexon on the growth of Pseudomonas fragi Bk_9.—The growth pattern of *P. fragi* Bk_9 on Seubert's medium [13] containing 0·5 per cent glucose with or without Dexon is shown in Fig. 3. When glucose alone was present the culture attained the stationary phase 14 to 16 hr after inoculation and the generation time as calculated from Fig. 3 is 3·5 to 4·0 hr. When

TABLE I

Effect of Dexon on oxidation of nitrite by Nitrobacter winogradskyi

Dexon conc. μ mole	Oxygen uptake (μl)	Per cent increase
0·0	446	..
0·1	525	17·7
0·2	592	32·7
0·4	493	10·5
1·0	456	2·2
2·0	492	11·2
12·8	576	29·1
25·6	538	20·6

Nitrite concentration .. 50 μ moles
Dry weight of the cells .. 8·5 mg/flask
Time of oxygen uptake .. 180 min.

TABLE II

Residual levels of Dexon (ppm) in soil

Depth of soil collection (cm)	75 days		96 days	
	Dexon		Dexon	
	100 ppm	200 ppm	100 ppm	200 ppm
10	3·46	9·50	0·21	0·45
20	5·55	8·02	0·26	0·17
30	4·29	12·38	0·46	0·20
Mean	4·43	9·96	0·31	0·27

Dexon (0·01 per cent) was added to this culture the generation time increased to 7 to 7·5 hr and bacteria attained the stationary phase only at 26 hrs after inoculation. This is suggestive of the delay in the utilization of glucose by this organism in the presence of the fungicide.

Breakdown of Dexon in soil.—When soil samples that were treated with Dexon were extracted and analysed for the presence of DMPDA we found that DMPDA is formed in low levels in the soil. We also observed that sterile soil when incubated with Dexon showed no DMPDA, whereas soils

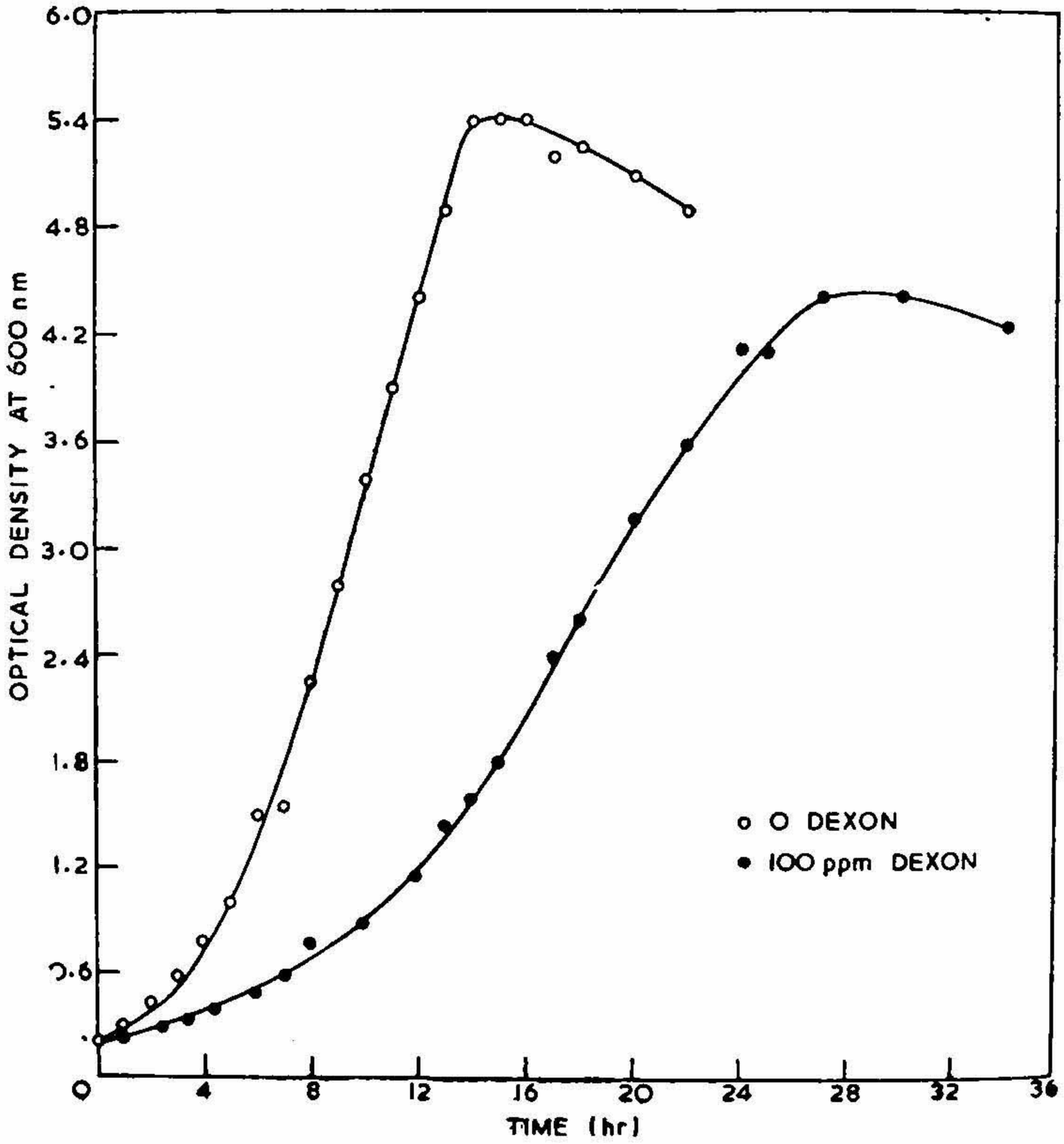

FIG. 3. Growth pattern of *Pseudomonas fragi* Bk_9 on Seubert's medium (0·5 per cent glucose) with and without Dexon.

inoculated with *P. fragi* Bk_9 showed rapid accumulation of DMPDA proving thereby that DMPDA is formed as a result of microbial action.

Effect of Dexon and DMPDA on the growth of Pythium.—Hills and Leach [11] have reported the specificity of Dexon on *Pythium* and its biological activity. When we used *Pythium aphanidermatum* as a test organism we got a linear relationship between the fungicide concentration and the reduction in the growth of the organism. Both in solid and liquid media, 15 ppm Dexon inhibited linear growth 50 per cent which is less than the concentration

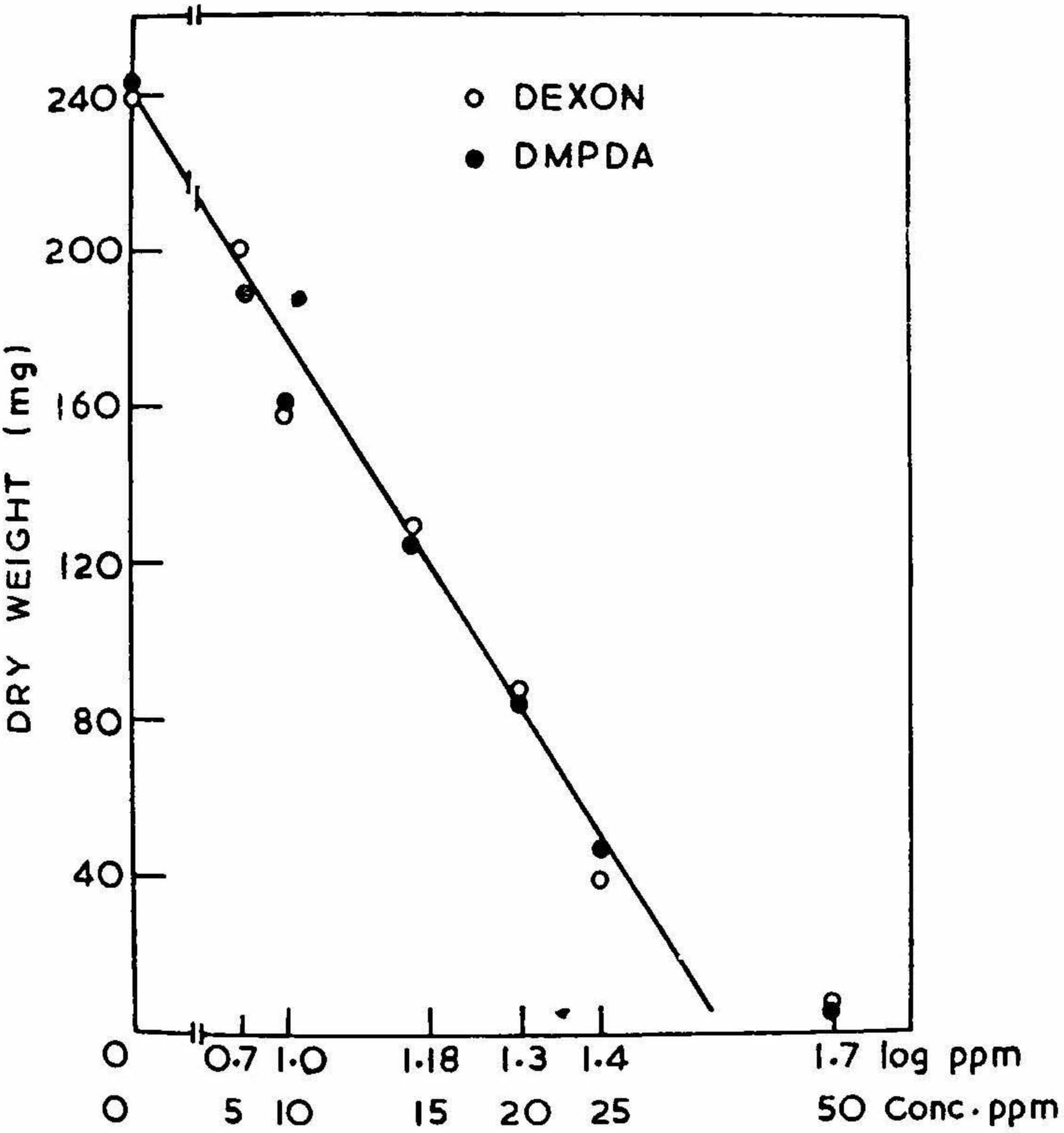

FIG. 4. Effect of concentration of Dexon and DMPDA on the growth of *Pythium aphanidermatum* in liquid medium.

calculated for *P. ultimum* [11]. Since DMPDA is the first breakdown product of Dexon, it was considered worthwhile to study whether or not DMPDA has any effect on the growth of *Pythium*. Interestingly we found that DMPDA is as toxic as Dexon therebeing a direct relationship between concentration and extent of inhibition (Fig. 4). However in solid media much higher concentrations were required to have the same effect. This was found to be true only in the case of DMPDA but not Dexon (Fig. 5) which may be due to differences in the affinity for and ability to diffuse in the agar gel.

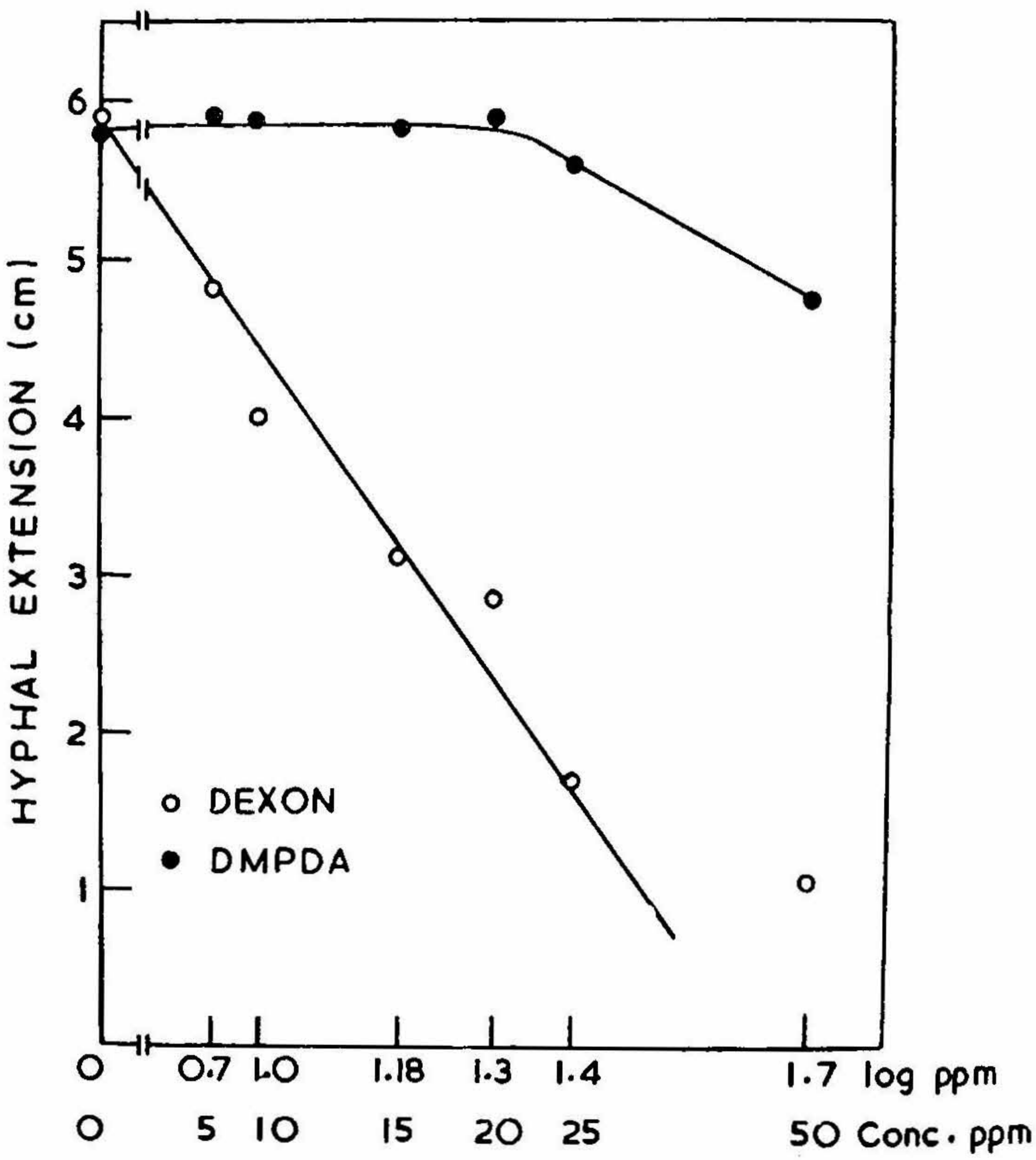

FIG. 5. Effect of concentration of Dexon and DMPDA on the growth of *Pythium aphanidermatum* on solid medium.

This finding is considered significant when viewed from the mode of action of certain other classes of pesticides. For example, the organo phosphorus pesticides are converted to their oxygen analogues which are more toxic than the parent compounds [14–16]. Dexon provides a situation where a metabolite formed by some other member of the community is equally toxic to the target organism.

Discussion

The use of pesticides in the fight for disease control is inevitable. What ever be the mode of application, the pesticides ultimately reach the soil Apart from disease control a proper comprehension of the influence of these chemicals on non-target organisms and their processes in soil is important.

Dexon did not reduce the plate counts of bacteria, actinomycetes of fungi. However, the fungicide was earlier observed to inhibit both soil respiration and the breakdown of organic matter in soil [17]. We have also reported the inhibitory effect of Dexon on certain soil ' enzymes [17 *a*]. This suggests that mere enumeration of changes in soil population may be of dubious value for evaluating the behaviour of pesticides in soil. Eventhough Dexon inhibits several soil processes the effect might last only for a short time as the disappearance of the fungicide is fast under local conditions [18].

The fact that Dexon inhibited nitrite oxidation in soil but not by the resting cells of *Nitrobactor winogradskyi* suggests that there may be present in soil a nitrifying component that is not autotrophic. Reports have appeared in the recent times [19–21] about the ability of several bacteria and fungi capable of heterotrophic nitrification. The two modes of nitrification may vary in their relative sensitivity to the presence of biocides in the environment. In the case of Dexon it might be that the heterotrophic process that is affected.

The detoxification of environmental chemicals can be brought about by several biological processes like oxidation, reduction, hydroxylation etc. [14, 22]. Dexon, for instance, is reduced to N, N-dimethyl-*p*-phenylene-diamine which is equally toxic to the target organism. This opens up a new vistas in studying the microbial interactions. It is felt pertinent to mention here that DMPDA is also formed from certain azodyes in mammalian systems [23].

This compound (DMPDA) causes several physiological changes like increase in ceruloplasmin levels (suggestive of liver disorders) when intro-

duced into animals [24]. The bacterial isolate we have studied metabolizes DMPDA poorly if at all. This makes it all the more interesting to follow the fate of DMPDA in soil and plants. There studies are at present in progress.

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