EFFECT OF BACTERIA STIMULATED IN SOIL TREATED WITH THE INSECTICIDE SEVIN (N-methyl-1-naphthyl carbamate) ON THE WILT PATHOGEN FUSARIUM OXYSPORUM f. VASINFECTUM IN CULTURE AND ITS PATHOGENIC POTENTIALITY IN SOIL

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INTRODUCTION

The preferential stimulation of saprophytic microorganisms in the soil following the application of insecticides or herbicides might restrict the growth of pathogens and stimulate plant growth. Warren *et al* (1951) stated that an actinomycete inhibitory to *Sclerotium rolfsii*, sacc. & *Rhizoctonia solani*, Kuhn became dominant in unsterile soil after spraying the plant with 2, 4,--D. Altman and Tsue (1965) isolated from fumigated soils a *Pseudomonas* sp. which could stimulate seed germination and seedling growth of sugar beets.

Application of the insecticide sevin (N - methyl - 1 - napthyl carbamate) to the soil stimulated certain strains of *Bacillus megaterium* and pseudomonads (Elwan & Khodair, 1969b). These strains were resistant to sevin ; one of them (*B. megaterium*, 2) utilized it as only carbon and nitrogen source. Some of these strains depressed the activity of *Azotobacter* (the authors, 1969c) and *Rhizobium* (the authors 1969d) in the soil. The aim of the present investigation is to reveal the nature of effect of these bacteria on *Fusarium oxysporum. f. vasinfectum* which causes cotton wilt.

MATERIAL AND METHODS

Microorganisms & host plant : The bacterial strains stimulated in the soil treated with sevin were *Bacillus megaterium* strains 2, 9 & 11 and pseudomanad strains 6, 7, 10 & 13 (the authors 1969b). The pathogen *Fusarium oxysporum f. vasinfectum* and the seeds of the susceptible host (menoufi cotton variety)

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were kindly supplied by Dr. Y.A. Youssef of this department. The pathogen showed 100% pathogenicity to the supplied host.

Preparation of inocula : A *Fusarium* mat of 7 days old in Czapek's liquid medium was shaken with few glass beeds in sterile distilled water to give a homogenous suspension. In culture work one ml. of this suspension served as inoculum for 20 ml. sterile medium in flasks of 250 ml. capacity. In soil work, 5 mls. served as inoculum for every 100 gm soil.

In culture work 1 ml. of 48 hrs. homogenised growth of bacteria in Czapek's liquid culture served as an inoculum for 20 ml. sterile medium in flasks of 250 ml. capacity. In soil work bacterial cells were sedimented by centrifugation, washed in physiological saline and their original volume restored by distilled water ; 5 mls. of this suspension served as an inoculum for every 100 gm soil.

Effect in culture : This has been carried out both on solid & in liquid media. For studying the effect on agar plates, a *Fusarium* disc (7 days old) was placed in an inverted position in the middle of a Czapek's agar plate and one loopful of an actively growing 18 hours old, liquid bacterial culture was inoculated at the periphary of the plate, so as 2 bacteria could be tested for in the same plate. Inoculations were made according to the following :

(*a*) The pathogen and the bacteria inoculated at the same time.
(*b*) The pathogen inoculated 2 days before the bacteria.
(*c*) The pathogen inoculated 5 days before the bacteria.
(*d*) The bacteria inoculated 2 days before the pathogen.
(*e*) The bacteria inoculated 5 days before the pathogen.

For studying the effect in liquid medium, *Fusarium* mycelial dry weights were the criteria for the effect. Three flasks were inoculated for each treatment. The inoculum was as above mentioned. Inoculations were made according to the following :

(a) Pathogen and bacteria inoculated at the same time.
(b) Bacteria inoculated 2 days before the pathogen.
(c) Bacteria inoculated 5 days before the pathogen.

Control flasks were prepared by inoculating 3 flasks with the fungus alone. The inoculated flasks were incubated at 30 C for 7 days after inoculating the pathogen. For collecting fungal mycelia filtration was made through Whatman filter papers No. 541 which were previously dried at 70-75 C for 24 hours, left to cool in a desiccator and weighed. Several consecutive washings were made that no apparent sign of bacterial growth was observed in the residue ; bacterial cells passed through the filter paper during washing and only the fungus

remained. When bacterial scum was observed no fungus mat was seen growing, filtration was not made and fungal growth was considered as zero. The filter paper containing the residue was dried in an electric oven at the above mentioned temperature (Cochrane 1963) for 24 hours, left to cool in a desiccator, and then weighed. Some flasks of both the treatments and their respective controls were photographed before filtration.

Effect in soil : The criteria for the effect in soil were the pathogenicity of *Fusarium* and the development of the susceptible host.

The pathogenicity was investigated by cutting transverse and longitudinal hand sections to observe browning and plugging of the vessels & by examining microtome stained sections to observe the occurrence of the pathogen, and by examining morphological symptoms (mosaic appearance, wilting of leaf margin and falling of cotyledonary leaves). Representative plants were surface sterilized bisected and left on Czapek 's agar plates. Other representatives were left in jars containing some water. *Fusarium* growth on the medium or on the plants in the humid atmosphere, indicated at least the entery of the fungus.

The developmental criteria of the host were the length of the primary root and the hypocotyl, both the length and width of the cotyledonary leaf, and the numbers of both lateral and adventitious roots per plant.

The experimental soils used for sowing with cotton seeds were :

(a) Soil.

(b) Soil inoculated with the pathogen alone.

(c) Soil inoculated with bacterium alone.

(d) Soil inoculated with both the pathogen and the bacterium.

Soil alone served as a control for development & soil with the pathogen alone as a control for pathogenicity & soil with the bacterium alone as a measure for its effect on development, and soil with both the pathogen and the bacterium as a measure of the effect of the latter on the pathogenic potentiality. Porous pots of about 700 gm capacity were used. In each treatment 10 pots were sown, each by 7 (48 hours soaked) seeds. Seeds were covered with about 2 cm. depth of soil. The pots were irrigated daily with equal volume of water at optimum conditions (temperature about 30 C) for 3 weeks.

RESULTS

In culture, *B. megaterium* strains 2, 9, & 11 inhibited the development of the pathogen (Table 1). Inhibition was evidenced by appearance of clear zone of inhibition on agar plates (e.g. Fig. 1), and absence of fungal growth in liquid medium (Table 2 & Fig. 2). On agar plates, when the bacteria were

Table 1 : Responses of *Fusarium oxysporum f. vasinfectum.* on agar plates to bacteria stimulated in soil treated with the insecticide sevin. (+) clear zone of inhibition ; (±) flattening or disintigration of hyphae ; (—) negative effect.

Bacteria	Inoculations of Bacteria and Fusarium				
	At the same time	Bacteria 2 days before Fusarium	Fusarium 2 days before bacteria	Bacteria 5 days before Fusarium	Fusarium 5 days before bacteria
B. megategium, 2	(—)	(—)	(—)	(—)	(—)
B. megaterium, 9	(—)	(—)	(—)	(—)	(—)
B. megaterium, 11	(+)	(+)	(±)	(+)	(+)
Pseudomonad sp., 6	(±)	(±)	(±)	(±)	(—)
Pseudomonad sp., 7	(±)	(±)	(±)	(±)	(±)
Pseudomonad sp., 10	(±)	(±)	(±)	(±)	(—)
Pseudomonad sp., 13	(—)	(±)	(±)	(±)	(—)

Table 2 : Responses of *Fusarium oxysporum*, f. vasinfectum in liquid medium to bacteria stimulated in soil treated with the insecticide "sevin" *B. megaterium* strains caused complete inhibition.
(—) indicates significant decrease.

Bacteria :	Mean mycelial dry weights (mgm.) in liquid medium inoculated with bacteria and *Fusarium.*		
	At the same time	Bacteria 2 days before	Bacteria 5 days before
Pseudomonad sp., 6	72.6	78.5	32.5 (—)
Pseudomonad sp., 7	61.8 (—)	74.1 (—)	72.7 (—)
Pseudomonad sp., 10	86.1	82.8	75.1 (—)
Pseudomonad sp., 13	79.1	89.1	71.2 (—)
Control (*Fusarium* alone)	84.4	109.2	112.6

inoculated 5 days before the fungus, its inhibition was sharp and distinct as compared to other appearances (Fig. 1).

As for the strains of the pseudomonads, the effect exerted on the fungus was not equally pronounced. Flattening of the fungus on the sides near to the bacteria or dying out and disintegration of the fungal hyphae beyond the line of contact, were the inhibitory signs observed (Table 1).

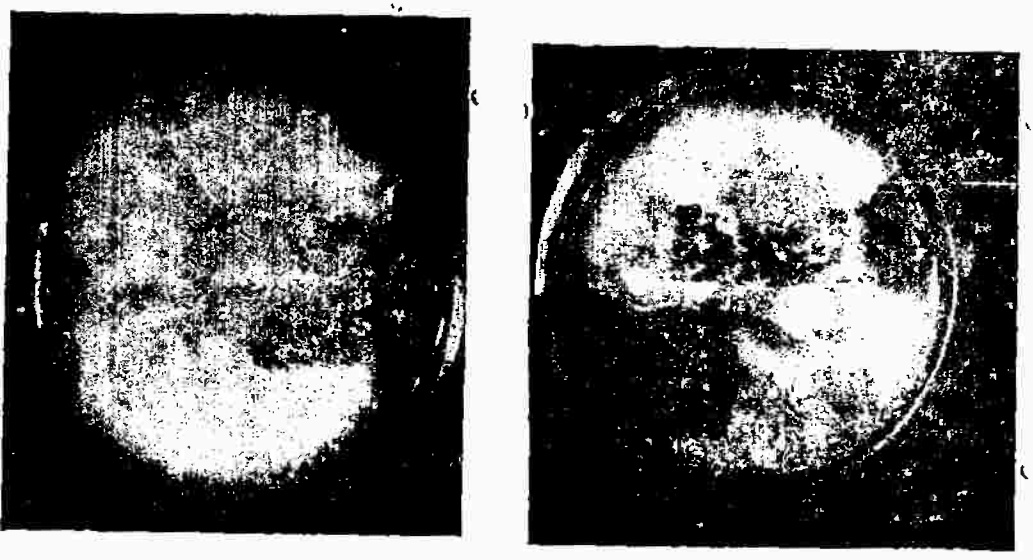

Fig. 1 — Representative plates for effect of bacteria on Fusarium which was inoculated 5 days before (right plate) or 5 days after (left plate) bacterial inoculation. B. megaterium, 9 (right side in every plate) inhibited Fusarium whereas Pseudomonad, 9 (left side in every plate) did not.

In liquid medium (Table 2) pseudomonad strain 7, produced significant inhibitory effect in all treatments, whereas strains 6, 10, & 13 exerte this effect only when bacterai were inoculated 5 days before the fungus. Negative results were even sometimes recorded in case of these strains (Table 1). *B. megaterium* strains gave definite and evident inhibition of the pathogen in all treatments made (e.g. Fig. 2). They were not tabulated in Table 2, because no fungal growth was observed. This is also why *B. megaterium* stranis only were inoculated in the soil togheter with the pathogen. Results showed that pathogenicity criteria were observed in both controls (treated with pathogen alone) and treatments. The variation in severity of symptoms (e.g. Fig. 3) was external. All the plants were definitely infected.

Tadle 3 : Effect of bacteria stimulated In soil treated with the insecticide sevin on some developmental criteria of cotton plant in presence and absence of *Fusaraim oxysoporum* f. vasinfectum: (+) & (—)indicate significant increase & decrease, respectively.

Control and treatments (soil inoculated with:)	Mean length (cm.) of :—			Mean width of cotyledonary leaf (cm.)	Mean No: of :—	
	Primary Root	Hypocotyl	Cotyledonary leaf		lateral roots per plant	Adventitious roots per plant
Not inoculated (control) :	6.51	7.32	3.64	2.01	5.45	1.98
B. megaterium, 2	6.54	9.06 (+)	3.22 (—)	1.61 (—)	8.08 (+)	0.96 (—)
B. megaterium, 9	7.30	10.20 (+)	3.35 (—)	1.66 (—)	10.24 (+)	2.35
B. megaterium, 11	7.09	9.03 (+)	3.37 (—)	1.64 (—)	7.64 (+)	1.18
Fusarium alone (control)	7.67	7.77	3.20	1.65	5.19	2.15
B. megaterium, 2 + Fusarium	5.17 (—)	6.27 (—)	2.89	1.52	3.67	2.73
B. megaterium, 9 + Fusarium	7.38	6.89	3.10	1.47 (—)	7.68	3.57
B. megaterium, 11 + Fusarium	4.77(—)	6.93	3.11	1.53	5.27	1.33

Fig. 2 — Representation to show the inhibition of Fusarium development (right) by B. megaterium, 2 as compared to the control (Fusarium alone, left). The fungus and the becterium were inoculated in the treatment flask at the same time.

Inoculation of *B. megaterium*, strains 2 and 11 but not strain 9, together with *Fusarium oxysporum f vasinfectum* significantly decreased the length of the primary root of the cotton seedlings. This did not happen when bacteria (as control) were inoculated alone in the soil. Significant increases in numbers of lateral roots when bacteria alone were inoculated in the soil, did not remain significant when soil was inoculated with both the bacteria and the pathogen together..

DISCUSSION

Bacteria stimulated in soil treated with the insecticide sevin have exerted suppressive effects on *Azotobacter* and *Rhizobium* (the authors 1969c & d). This was considered a sign of deterioration of the fertility standard of the soil. In this investigation the inhibition of the wilt pathogen *Fusarium oxysporum* f. vasinfectum in culture was thought of importance in controlling this pathogen biologically in the soil. Results however, have shown that the behaviour in culture differed from the behaviour in soil. Various factors might be involved in this respect of which the "microbiological factor" is suggested to be the most

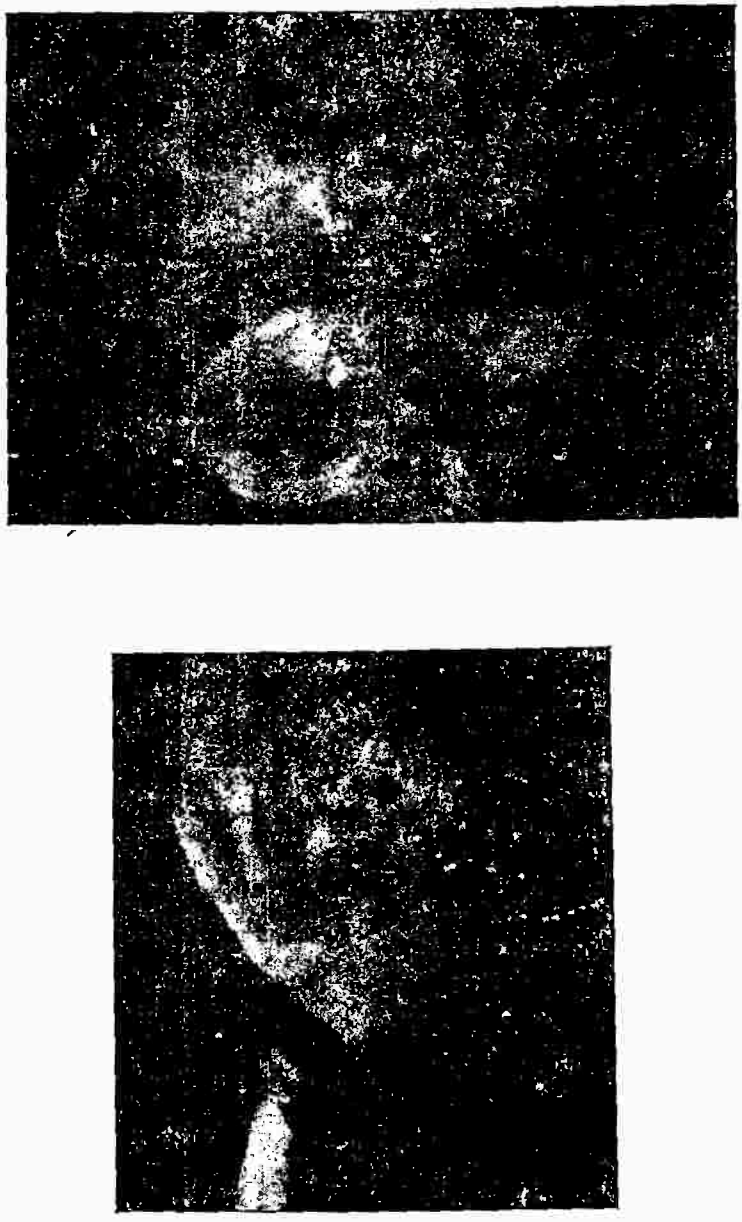

Fig. 3 — Menoufi cotton seedlings raised for 3 weeks in soil inoculated with the pathogen alone lower and the pathogen together with Bacillus megaterium, 9 upper. Difference in severity is external, both showed internal symptoms of infection.

important. Inoculation of the sevin - stimulated bacteria in the soil might have resulted in the dominance of some microorganisms which are antagonistic to them. Therefore, these dominating bacteria might have decreased the activity of antagonising the pathogen in the soil by the sevin - stimulated bacteria. In evidence of the importance of the "microbiological factor" is the recorded ever-changing (dynamic) nature of the microbial equilibrium in the soil in response to applying the insecticide sevin (the authors 1969a), and probably in response to applying any additive to the soil.

The variability of behaviour of antagonistic microbes in culture from that in soil has been demonstrated also by Mostafa and Gayed (1953). They observed that *Trichoderma viride* was antagonistic to *Fusarium vasinfectum* in culture whereas it failed completely to exert any antagonistic effect towards *Fusarium* pathogenicity to cotton.

The observed variation in the degree of severity of wilt symptoms being less drastic in the presence of the investigated *B. megaterium* strains, might point at the importance of the "inoculum size" as a factor responsible for the observed results. The evaluation of this factor needs further investigation.

B. megaterium strains induced significantly adventitious root formation when inoculated alone in the natural soil whereas this induction was not significantly observed when these strains were inoculated in soil together with *Fusarium*. Although the importance of this finding was not reflected on the occurrence of the pathogenicity criteria, yet it might perhaps have any other physiological value (the degree of severity of symptoms to be mentioned in this respect). The authors suggest that executing tests for biological control of plant pathogens in culture only are by no means integral or adequate.

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SUMMARY

Three strains of *Bacillus megaterium* and four ones of Pseudomonad spp, stimulated in sevin treated soil, were tested for their effects on the development of *Fusarium oxysporum* f. *vasinfectum*, in culture, and its pathogenic potentiality to the susceptible Menoufi cotton plant, in the soil.

B. megaterium strains inhibited the fungus in Czapek's liquid and agar media. On the other hand, pseudomonad strains exerted much less inhibitory effect. Results of inoculation of *B. megaterium* strains, together with the pathogen in the soil in which Menoufi cotton plants were raised, were not similar to the results obtained in culture. In all the raised plants the pathogenicity criteria were observed. These were the browning & plugging of vessels, the occurrence

of the pathogen in transverse sections of the plant, the morphological symptoms namely mosaic appearance, wilting of the margin and falling of the cotyledonary leaves, and the development of *Fusarium* hyphae on the incubation of surface-sterilized and bisected plants. However, the severity of the symptoms was much less reduced.

On the other hand, significant differences were recorded in some morphological determinations (e.g. adventitious root formation) but these were not reflected on the occurrence of the pathogenicity criteria ; they probably were the cause of the reduction in the severity of symptoms. The authors suggested that carrying out tests for biological control of plant pathogens in culture only are by no means integral or adequate.

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