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STUDIES ON EFFECTS OF BENOMYL (BENZIMIDAZOLE) ON NON-TARGET MICROFLORA OF A TOMATO CROPPED SOIL.

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ABSTRACT

The effect of benomyl on the microflora of a tomato cropped soil was investigated. Benomyl applied as soil treatments in micro pots at 25 ppm, 50ppm, 75ppm, 100ppm and 125ppm levels lowered fungal counts while there was a corresponding increase in total bacterial count with increasing benomyl concentration. Studies were also conducted to observe the effect of varying concentrations of benomyl on the population of *Pseudomonas* species in tomato soil. *Pseudomonas* counts decreased with increasing concentration of benomyl both in culture and soil treatments. It was concluded that the use of benomyl in cultivation of tomato may not predispose the crop to Pseudomonad infections.
Key Words: Benomyl, non-target microflora, tomato soil.

INTRODUCTION

Tomato (*Lycopersicum esculentum*) is an economically important crop all over the world. Tomato fruit is eaten raw in salad or made into puree, ketch up, soup or powder in canning industries. Inspite of the varied uses and wide acceptance, commercial production of tomato in West Africa is limited by diseases associated with high rainfall and humidity. Tomato diseases include wilts, leaf spots, blights, fruit spots and rots (MacNab *et al.*, 1983). Four common wilts of tomato plant are *Fusarium* wilt, *Verticillium* wilt, Walnut wilt and bacteria wilt. *Fusarium* wilt and some other fungal infections of tomato are controlled by using fungicides. Benomyl (methyl – 1 (butyl carbomyl)$^{-2}$ bezimidazole carbamate) has been successfully employed as a systemic fungicide for the control of various plant diseases (Biehin and Dimond, 1970). According to Chanon and Thomson (1973) fusarium wilt of potted tomato was controlled by soil drenches of benomyl. A shift in soil borne pathogen populations following treatment with selective fungicides may result in control of one disease while another disease is induced. Williams and Ayanaba (1975) provided evidence of non-target effects of benzimidazole fungicides in the field. A positive relationship between a field increase in incidence of *Pythium* stem rot of cowpeas on application of benomyl was reported. Pseudomanads have been associated with diseases of tomato plants (Ram, 1987). Pseudomonad diseases of tomato include bacteria wilt disease of tomato caused by *Pseudomonas solanacearum*, tomato pith disease caused by *P. corrugata* (Sesto *et al.* 1996) and bacteria speck caused by *P. syringae* (Amer *et al.*, 1996). It has been reported that *P. solanacearum* is antagonized by many soil microorganisms (El-abyad *et al.*, 1996). If the antagonists are wiped off by any chemical or biological agent, the population of the pathogen is likely to increase in soil. It is important to know the effects of benomyl treatments on the population of pseudomonads as an increase in the population will predispose susceptible crops to infection.

This paper therefore presents information on changes in microbial population of tomato soil treated with different concentration of benomyl and response of pseudomonads to benomyl in culture medium and in tomato soils.

MATERIALS AND METHODS

Collection of samples and treatment with benomyl

Soil samples were collected from a vegetable farm in which tomato had been cropped for two years in Ilorin. Random samples were collected at 15cm depth, bulked and transported to the laboratory in polyethylene bags. The soil was air dried and passed through a 2mm sieve. Fifteen micropots (7.5cm diameter) were filled with soil at rate of 250g/pot. Benomyl treatments were applied to the soil at 25ppm, 50ppm, 100ppm and 125ppm levels. Each treatment was in triplicate.

Isolation and identification of microorganisms

The soil fungal and bacterial population was determined weekly using the soil dilution technique. The 10^{-5} dilutions were plated out in Nutrient agar (NA) for bacteria enumeration while 10^{3} dilutions were plated in potato Dextrose Agar (PDA) supplemented with 1% chloramphenicol for fungal counts. The NA plates were incubated at 37 $\pm$ 2 ^{0} c for 24 hours. The colony morphology of bacterial isolates on NA medium, Gram reaction and microscopic morphology were observed. Cultural features of fungal isolates on PDA and microscopic morphology were also employed in their identification

Response of Pseudomonads in soil to benomyl

Two sets of experiments were carried out. The response of Pseudomonads to benomyl in culture was observed by incorporating benomyl in Pseudomonas Selective Medium (PSM) at 100 ppm and 200ppm levels. A control set up had no benomyl (0ppm). Ten fold serial dilution of untreated soil samples were made. The 10^{-5} dilutions of soil were plated out in PSM-benomyl preparations using pour plate method.

In the second experiment soil in a set of pots were treated with benomyl

(100ppm) while a control set of pots had no benomyl treatments (0ppm). Soil samples (10g) were collected per pot after 7 days of treatment with benomyl 0 Pseudomonads were isolated from the samples in PSM using soil dilution technique. Plates were incubated at 37°c for 24 hours after which colonies were counted. Growth relationships between *Pseudomonas* isolates and *Fusarium oxysporum* were observed on plates of nutrient agar medium.

RESULTS AND DISCUSSION

Changes in fungal population of benomyl treated tomato soil over a period of four weeks is shown in Fig. 1. A general drop in fungal count was observed at second week of sampling in both control and experimental pots. This is probably a response to changes in availability of nutrients for fungal metabolism brought about by existing environmental conditions of micropots. It was however, observed that number of fungi in benomyl treated soils were lower than that of the control pots. The total fungal counts were found to reduce with increasing concentration of benomyl. This can be attributed to the broad-spectrum action of benomyl, a systemic fungicide that has been shown to have effects on many soil fungi.

Slight increases in fungal population were noticed by four weeks after treatment , an indication of reestablishment of fungal flora. It has been reported that benomyl breaks down rapidly in soil. Out of several soils investigated in one study, only one contained a residue of intact benomyl four weeks after application. Carbendazin, a major primary break down product of benomyl was fungitoxic but more of non-fungitoxic aminobenzimidazole was formed with passage of time (Kenneth, 1990).

Penicillium chrysogenum had the highest percentage frequency of occurrence at four weeks after treatment due to fungicidal action of benomyl on most of the other fungal flora. *Aspergillus flavus* and Curvularia sp were also encountered in the soil at four weeks after benomyl treatment (Figure 2).

Changes in total bacterial count of benomyl treated tomato soils are shown in Figure 3. The population of bacteria increased with increasing concentration of benomyl. The bacterial count could be as a result of microbial competitive or antagonistic interrelationships in the soil.

Since bacteria and moulds co-exist in the soil competing for food, inhibition of fungal flora by benomyl would make more nutrients available for the bacterial flora. Gram-negative short rods were abundant in the benomyl treated tomato soil.

In a second study designed to investigate response of Pseudomonads to benomyl, five *Pseudomonas* strains designated P_1, P_2, P_3, P_4 and P_5 were encountered in each of the two experiments carried out. Benomyl in culture medium and in soil lowered *Pseudomonas* counts (Tables 1 &2). Strains P_3, P_4 and P_5 exhibited high sensitivity to benomyl in culture as they only appeared on control plates. Strain P_5 was

however found in benomyl treated soil. Peeples (1974) stated that some fungi are sensitive to benomyl in culture but appear to grow in normal populations in benomyl treated soil. Another *Pseudomonas* strain P2, showed a varying response to benomyl in culture and soil. While the population of this Strain was reduced with benomyl in culture, the number increased slightly when benomyl was used as soil treatment. This is probably as a result of disrupted interrelationships between this isolate and fungal flora of the soil due to benomyl application.
Investigations on growth relationships of *Pseudomonas* isolates and *Fusarium oxysporum* showed that the Strain P_4 exhibited antagonism towards the *Fusarium* species in plate culture. Tu and Zheng (1994) made similar observations with a species of *Pseudomonas*. They demonstrated that the bacteria could offer significant control of *Fusarium* crown root rot.

CONCLUSION

Although an increase in total bacterial population of tomato soil was observed with increasing concentration of benomyl, strains of the *Pseudomonas* species were found to be lowered in numbers. Benomyl probably inhibits potential pathogenic Pseudomonads in soil. It can therefore be concluded that the use of benomyl as fungicide for control of fusarium wilt may not predispose the tomato plant to Pseudomonad infections.

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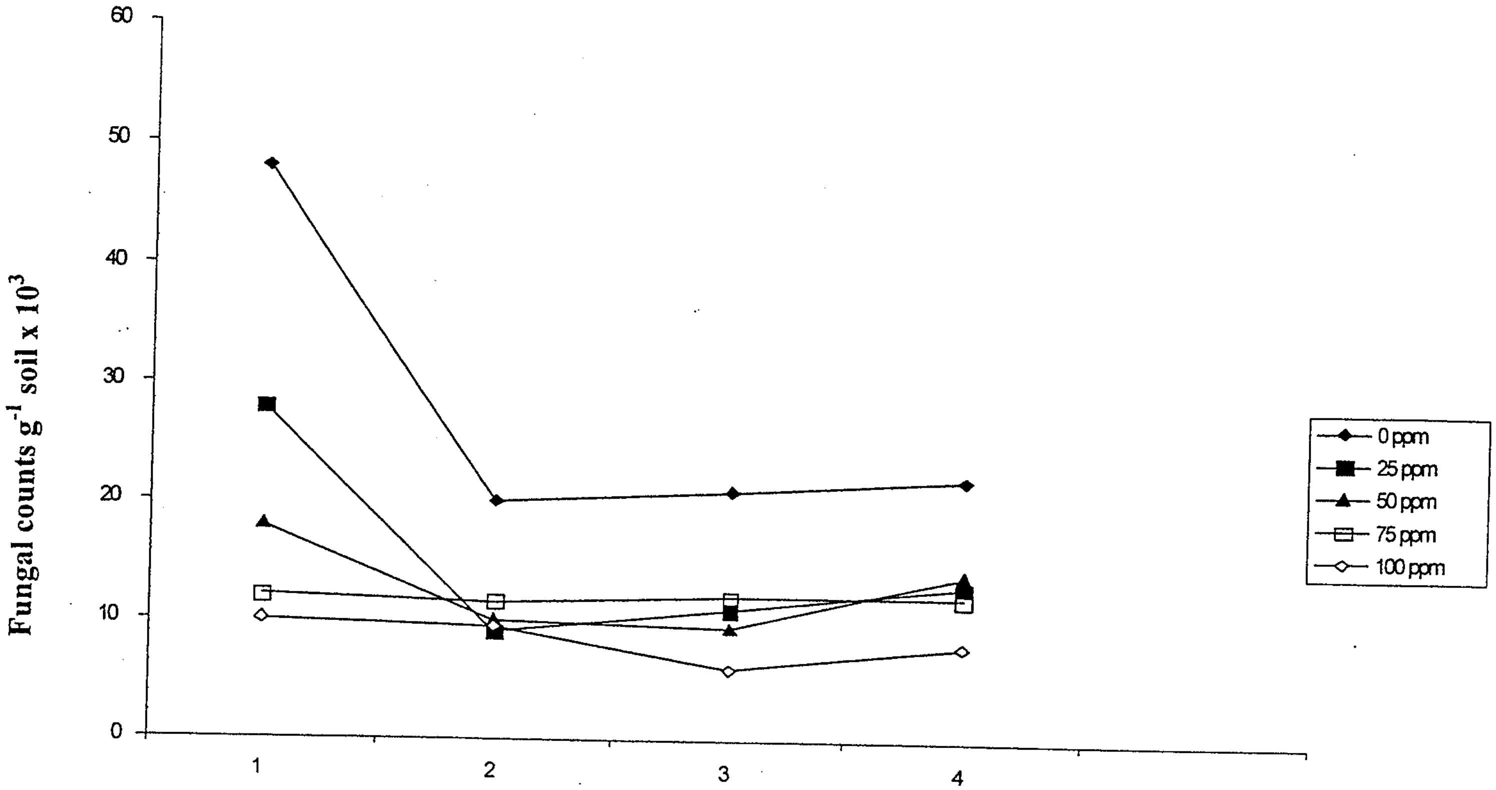

Weeks

Fig. 1.Changes in total fungal counts in tomato cropped soil treated with different concentrations of benomyl

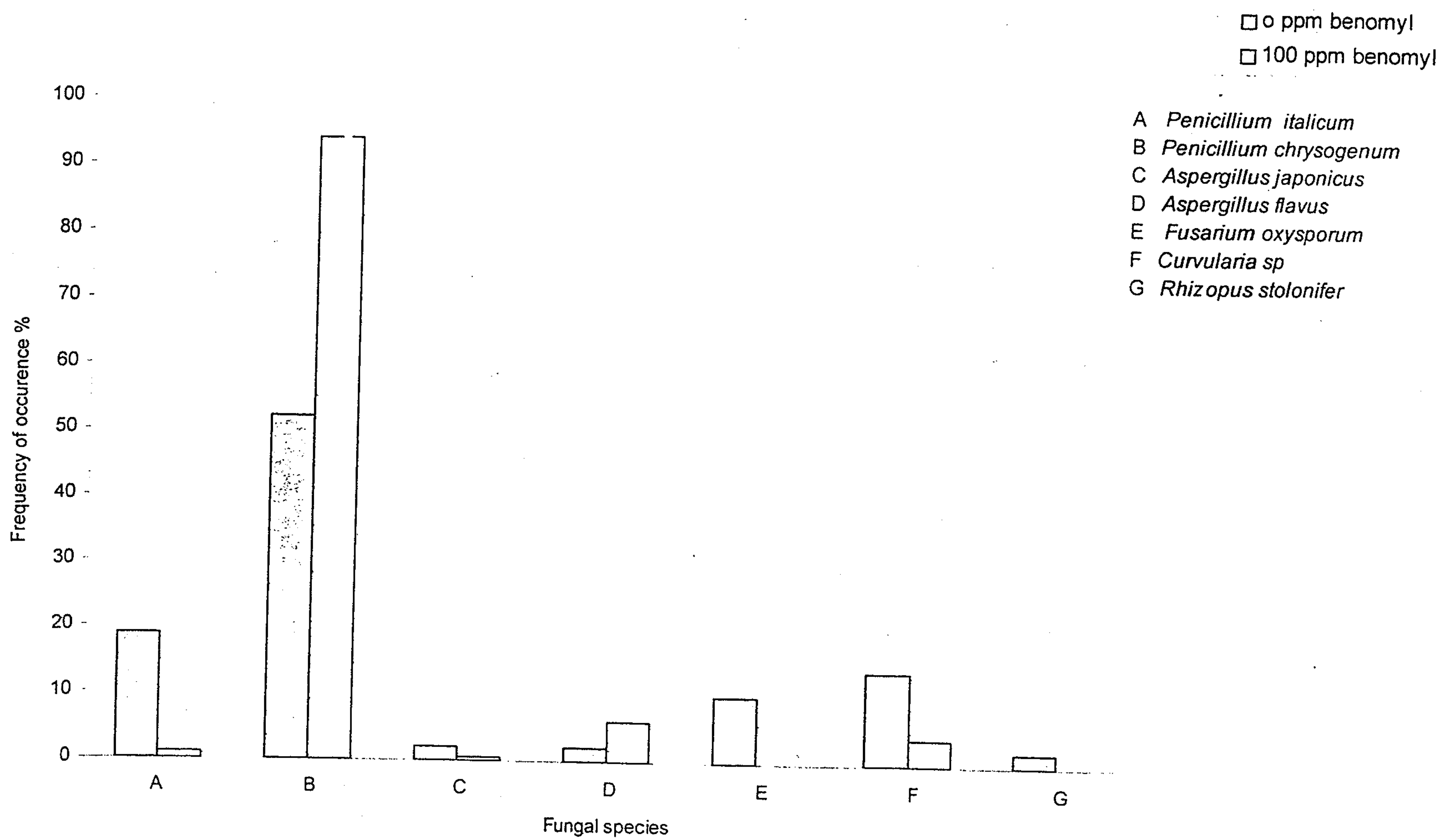

Fig. 2. Frequency of occurrence of Fungi in tomato cropped soils four weeks after benomyl treatment

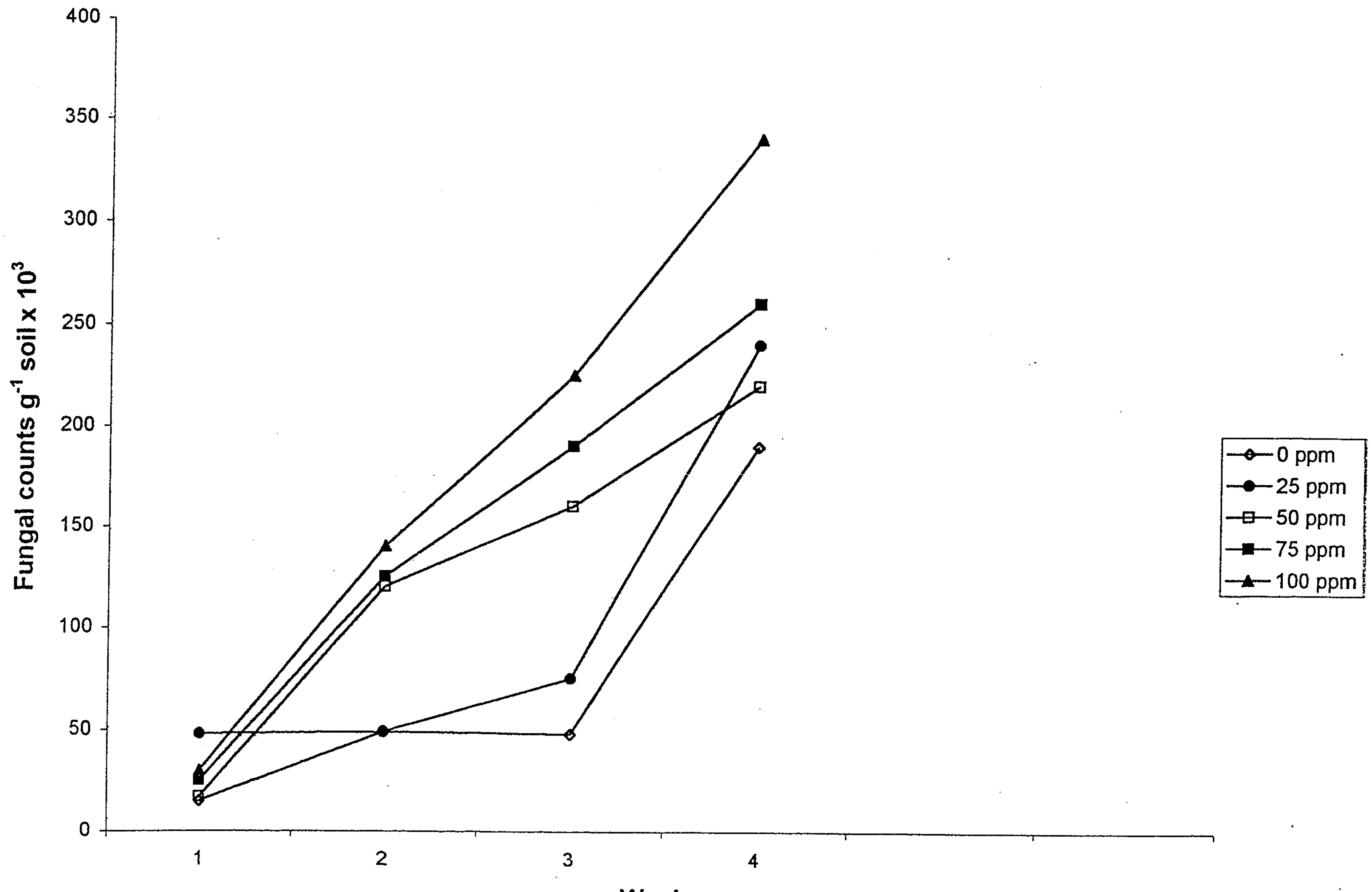

Fig.3.Changes in total bacterial counts in tomato cropped soil treated with different concentrations of benomyl

Table 1: Response of *Pseudomonas* species from tomato soil to varying concentration of benomyl incorporated in *Pseudomonas* selective agar medium.

Isolate Designation	Mean Pseudomonas count g^{-1} soil Benomyl concentration (ppm)		
	0	100	200
P^1	$6.7x10^4$	$3.3x10^4$	0.0
P^2	$13.3x10^4$	$3.3x10^4$	0.0
P^3	$10.0x10^4$	0.0	0.0
P^4	$3.3x10^4$	0.0	0.0
P^5	$6.7x10^4$	0.0	0.0
Total count	$40.0x10^4$	$6.6x10^4$	0.0

Table2: Response of *Pseudomonas* species in tomato cropped soil treated with benomyl

Isolate Designation	Mean Pseudomonas count g^{-1} soil Benomyl concentration (ppm)	
	0	100
P^1	$63.0x10^4$	$43.0x10^4$
P^2	$3.3x10^4$	$6.0x10^4$
P^3	$3.3x10^4$	0.0
P^4	$47.0x10^4$	0.0
P^5	$57x10^4$	$43x10^4$
Total counts	$173.6x10^4$	$132.0x10^4$