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Response of Microbial Population and Enzyme Activities to Fungicides in Potato Field Soil

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The response of soil microflora and the enzymatic activities to fungicides (benomyl, copperoxychloride and mancozeb) in potato field soil has been studied. The fungal and bacterial populations of soil dropped significantly following application of fungicides and throughout the study period remained lower than the control. Fungicides also inhibited enzymatic activities. Inhibitory effect was more pronounced in case of dehydrogenase and phosphatase than the urease activities.

Key Words: **Potato, Fungicide, Fungi, Bacteria, Enzymes**

Introduction

Any compound which alters the number or activity of microorganisms could affect the soil biochemical processes, and ultimately the soil fertility and plant growth (Kruglov 1990, Banerjee & Dey 1992). Although during recent times the use of fungicides in potato crop has increased by many-folds, the effect of fungicides on microbes and their activities has not received ample attention. Present study deals with the response of fungal & bacterial populations and enzymatic activities, (dehydrogenase, urease ' and phosphatase) to fungicides in potato field soils.

Materials and Methods

The study was conducted under terrace land system in the farms of Central Potato Research Station (CPRS), Shillong. Disease free tubers of 'Kufri jyoti' were sown in the last week of March. Five 30 M^2 plots were taken for each treatment and the control, all set out in a fully randomized design. The fungicides recommended for potato and commonly used by growers of this region were used. The chemical names, commercial names, recommended doses and manufacturers of the fungicides were as follows : Benomyl, Methyl 1-(Butyl carbomyl)-2-benzimidazol carbamate, Benlate (0.37 kg/ha), Du Pont, USA; Copper oxychloride, Blitox-50 (7.4 kg/ha), Rallis India; and Mancozeb, Zinc ethylene bisdithio carbamate, Dithane M-45 (2.0 kg/ha), Indofil Ltd., India. Pretreatment soil samples were

collected and fungicides sprayed 20 days after emergence of plants. Subsequent samples were collected on 15 days interval. Soil plate method was used to assess fungal propagules developing on rose bengal agar medium while dilution plate method was used to estimate bacterial population on nutrient agar medium. Petri plates for fungi were incubated at 25°C for seven days and for bacteria at 30°C for 24 hr. Dehydrogenase activity of soil was determined as suggested by Casida (1977). One gram of soil sample

Table 1 *Response of microbial population and enzymatic activities (per gram dry soil) on application of various fungicides*

Fungicides period (days)	Fungi ($\times 10^3$)	Bacteria ($\times 10^5$)	Dehydrogenase (µg TPF/24hr)	Urease (µg NH^+_4/3hr)	Phosphatase (µg p-nitrophenyl/hr)
Control					
0	51.8	40.2	48.0	131.0	270.0
15	53.2	51.1	56.7	133.2	281.2
30	59.6	65.6	78.2	146.8	297.6
45	78.7	172.4	189.7	168.5	326.8
60	73.9	170.0	172.6	166.3	320.4
Copperoxychloride					
0	51.8	40.2	48.0	131.0	270.0
15	30.0	27.0	49.1	127.2	247.0
30	26.4	30.1	36.2	115.2	230.5
45	39.2	103.0	52.7	136.1	232.0
60	54.7	102.4	69.8	136.0	276.8
MANCOZEB					
0	51.8	40.2	48.0	131.0	270.0
15	32.1	26.3	47.7	126.7	250.7
30	27.1	33.0	35.6	120.0	223.8
45	43.6	106.1	53.6	129.0	236.1
60	51.2	105.0	67.7	130.6	277.8
BENOMYL					
0	51.8	40.2	48.0	131.0	270.0
15	50.3	51.0	54.6	132.7	278.6
30	30.0	55.0	50.0	116.2	263.1
45	25.8	150.0	71.7	123.6	226.7
60	49.1	146.5	72.0	120.5	256.9
L.S.D (P = 0.05)	7.9	21.6	18.3	9.6	13.6

taken in dry test tube was treated with 0.1 g $CaCO_3$ and 1ml of 1% triphenyl tetrazolium chloride (TTC). The content of each tube was mixed well, plugged with rubber stopper and incubated at 30°C for 24 hr. As a result of dehydrogenase activity the TTC. was reduced to triphenyl formazan, which was extracted with methanol and volume made upto 50ml mark. The optical density of pink-coloured filtrate was determined at 485 nm by spectrophotometer (Hitachi 220). Urease activity was estimated by the method of McGarity and Myers (1967). One gram of soil was placed in 100 ml volumetric flask and to this was added 1 ml of toluene. Subsequently 10 ml of buffer (*p*H 7) and 5 ml of 10% urea solution were added. The flasks were shaken and incubated for 3 hr at 37°C. After incubation the content of flask was made upto 100 ml with distilled water and filtered. 0.5 ml of filtrate was placed in a 25 ml volumetric flask and to this was added 4.5 ml distilled water, 2.5 ml phenolate solution and 1.5 ml of sodium hypochlorite solution. After 20 minutes the volume was made upto 25 ml by adding distilled water and optical density of blue-coloured solution was measured spectrophotometrically at 630 nm. Phosphatase activity was measured by the method of Tabatabai and Bremner (1969). One gram of soil was taken in 50 ml conical flask and to this 4 ml of modified universal buffer (*p*H 6.5), 0.25 ml toluene and 1ml 0.115M disodium p-nitrophenyl phosphate hexahydrate were added. The flasks were stoppered and incubated at 37°C for 1 hr. After incubation of 1ml, 0.5M $CaCl_2$ and 4ml of 0.5M NaOH were added to each flask. The soil suspension was filtered and optical density of filtrate was measured in a spectrophotometer at 420 nm.

Results and Discussion

Fungicides spray resulted into a drop in the microbial population. Throughout the study period fungicides-treated plots harboured less population than the control (table 1). Similar results were reported by other workers (Colinas et al. 1994, Shukla et al. 1987). In most cases after initial drop in population a recovery was observed the recovery in case of bacteria being faster, which may be attributed to their tolerance to the action of fungicides (Kruglov 1990). It could be inferred that the adverse effect of fungicides was severe and prolonged for fungi. Among the three fungicides, benomyl did not reduce bacterial population severely. Liu and Hsiang (1994) also reported that benomyl had no effect on bacterial population. Colinas et al. (1994) found that fungicides drastically reduced fungal, bacterial and actinomycetes populations. Dehydrogenase and phosphatase activities were found inhibited on application of fungicides. Though urease activity was also reduced but the intensity was not so low as in the case of dehydrogenase and phosphatase. Gianfreda et al. (1994) reported that application of fungicides altered the urease activity in soil. Copper oxychloride and mancozeb were more inhibitory for enzyme activities as compared to benomyl. The soil dehydrogenase system probably consists of different enzymes or enzyme systems which have a role in the initial stages of oxidation of soil organic matter. The activity of phosphatase is an index of the activity of microflora involved in soil organic phosphate decomposition. In soil, urea is rapidly hydrolyzed to ammonium carbonate by urease activity resulting in formation of nitrate and ammonia. Contact fungicides like mancozeb and copper oxychloride may have more severe effect on

microbial population and activities, while benomyl a systemic fungicide does not have same mode of action as other two fungicides (Gruzdyev et al. 1988). That is why bacterial population and enzymatic activities were not reduced same way as in case of mancozeb and copper oxychloride. The studies indicated that the fungicides selected for the study had an effect similar to those caused by standard microbial inhibitors on soil fungal, bacterial population and enzyme activities.

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