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Biological Effects of Four Fungicides on Soil Microbial Population

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Abstract: The biological response of bacteria, actinomycetes, fungi and protozoan to four fungicides (Phenyl mercuric acetate, pentachloro-nitrobenzene, benomyl and captan) was investigated in a garden soil treated with three different rates of these fungicides. The microbial populations were estimated at different days after treatment using the standard dilution plate-count technique. Phenyl mercuric acetate completely inhibited the soil bacteria and fungi at all rates of application up till 33 Days After Treatment (DAT), after which recolonization of the soil occurred. The significantly ($p \geq 0.05$) highest bacteria population of 22.11×10 cfu g^{-1} and 16.03×10 cfu g^{-1} of actinomycetes population in soil was observed in the soil samples treated with benomyl at the application rate of 225.0 ìg g^{-1} and 63DAT when compared with that of untreated soil sample. Pentachloro Nitrobenzene (PCNB) gave significantly lowest ($p \geq 0.05$) population of actinomycetes (0.03×10 cfu g^{-1}) and protozoan (0.0×10 cfu g^{-1}) compared to all other treatments throughout the period of study. The actinomycetes population in the captan and ceresan treated soils sample increases with days after treatment. In general, fungi and protozoa were more susceptible to fungicides than bacteria and actinomycetes. Phenyl mercuric acetate and pentachloro-nitrobenzene were more toxic particularly to soil, micro organisms, compared to benomyl and captan. The significant effects of fungicides on soil microbial population is here in discussed.

Key words: Fungicides, microbial population, soil, application rate, actinomycetes, PCNB

INTRODUCTION

The application of pesticides in particular fungicides has become an integral part of crop production throughout the globe especially in the tropics where many pathogenic organisms abound. Several of these are used without following the recommended rate of application (Adebayo and Adebayo, 2006). Although intended to protect crops from plant pathogens, they go beyond this intended role by altering the mycoflora of soil ecosystems. In the fungicide control of pathogens, it is important to avoid serious injury to a great variety of microbes whose functions are vital to the crop producing power of the soil. It is very important to know the side effects of these fungicides on different forms of life inhabiting the soil (Ojo *et al*., 2006). The indiscriminate use of fungicides in the developing nations where there is little or no attention towards their usage may aggravate a disease situation rather than controlling it. This is a common occurrence with fungicides, which are usually applied to soil at high rates (Ojo *et al*., 2006). The resultant harmful effects of fungicides on microbial populations, parasites or competitors of the plant pathogens may be their elimination.

The treatment of legume seeds with Thiram, Spargon and Phygon before rhizobial inoculation decreased the weight of plant and nitrogen fixation considerably (Cremyl, 2006). Similarly, gammalin and phenylmercuric acetate were observed to be toxic to tropical status of rhizobia (Odeyemi and Ogunledun, 1983; Odeyemi *et al*., 2005). Furthermore, application of benomyl, captan and zineb at the recommended rates have been reported in India to have resulted in significant reduction in fungal population in the rhizosphere of some crops, whereas bacteria and actinomycetes population increased significantly (Rao and Sharma, 2001; Annapurna and Rao, 2002; Ekundayo, 2003). From the foregoing, it becomes imperative that special attention must be paid to the non-target effects of fungicides on microbial population so as to ascertain their specific effects in agricultural soils particularly in Nigeria.

MATERIALS AND METHODS

Collection of samples: Samples from top 15cm of a garden soil in the Teaching and Research Farm of Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria were collected in black polythene bags. The soil was sandy loam texture and had no history of pesticide treatment for over twenty years. The samples were air-dried and sieve through a 1.5mm sieve before use. The soil had a moisture context of 26.2% when fresh and

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1.9% when air-dried. The percentage moisture content of the soil was increased to 10% by adding distilled water during study.

Application of pesticides: The following commonly used fungicides: Pentachloro-Nitrobenzene (PCNB); benomyl, captan and ceresan were employed in this study: The fungicides were applied to 100g portions of soil at below recommended rate (R1); recommended rate (RII) and above recommended rate (RIII) respectively for each of the fungicides. In PCNB at 0.10 kg litre^{-1} or 12 g 100 g^{-1} of soil for RI; 0.20 kg litre^{-1} or 24 g 100 g^{-1} of soil; 0.30 kg litre^{-1} or 36 g 100 g^{-1} of soil. In benomyl at 0.14g/litre or 0.023 g 100 g^{-1} soil for RI; 0.37glitre or 0.045 g 100 g^{-1} soil for RII and 0.51g/litre or 0.068 g 100 g^{-1} soil for RIII; ceresan at 35 μg g^{-1} soil or 0.003 g 100 g^{-1} for RI; 50 μg g^{-1} soil or 0.005 g 100 g^{-1} soil for RII and 75 μg g^{-1} soil or 0.008 g 100 g^{-1} soil for RIII while captan was applied at 30 μg g^{-1} soil 0.004 g 100 g^{-1} for RI; 60 μg g^{-1} soil or 0.006 g 100 g^{-1} soil for RII and 80 μg g^{-1} soil or 0.009 g 100 g^{-1} soil.

A calculated amount of each fungicide was applied following the method of Ekundayo (2003) in which each fungicide was weighed into plastic cups containing 100g soil sample, thoroughly mixed together and moistened with sterile distilled water. The plastic cups were then covered with sterile aluminum foil to prevent contamination and later incubated at 30oC for 3 days. Thus, the period of expected toxicity of the pesticides to soil organisms was 3 days. Samples of each pesticide concentration were prepared in triplicate. The pesticide free weighted soil samples (controls) were moistened with sterile water and also incubated for 3 days. All treatments were replicated three times.

Microbial isolation techniques: The method of dilution-plate was used for estimating the population of each microbial group as employed by Ekundayo (2003). Each microbial group population was estimated just before fungicides treatment and at 3, 18, 33, 48 as well as 63 Days After fungicide Treatment (DAT) respectively.

Dextroses-il extract agar containing 10 litre, 1.0g dextrose, 0.5K_2HP0_4, 100mL soil extract and 15g agar were used for counting the actinomycetes. Asparagine-manitol agar containing per litre, 0.5g asparagines, 1.0g mannitol, 1.0gK_2Hp0_4. 0.2g $MgS0_4$, $7H_20$, 0.1g $CaCl_2$, 0.1g NaCl, 0.5g $KN0_3$ and 15g agar were used to estimate the bacterial density. Fungal number was determined with Potato Dextrose Agar (PDA) which consisted of 4g potato extract, 20g dextrose and 15g agar per litre of distilled water. The medium was acidified to PH 3.5 with acetic acid. Mannitol-soil extract agar containing, per litre, 5.0g mannitol, 0.5g K_2HP0_4, 100ml soil extract and 15g agar was used for assessing the population of protozoan

RESULTS

The results on the effects of different concentrations of some fungicides on the population of bacteria, actinomycetes fungi and protozoan in the soil at different days after treatment are presented in Tables 1-4 respectively. The result showed that Ceresan completely inhibited the population of soil bacteria at all rates of application up till 33 Days after Treatment (DAT), after which there was recolonization (Table1). In addition, the bacteria population decreased with the rate of PCNB, benomyl and captan but increased with the days of treatment (Table 1). The significantly highest (p= 0.05)

Table 1: Effect of different concentrations of fungicides on bacteria populations of soil at different days after treatment

Rate of application (μg g^{-1})			Mean *bacteria population* (cfu×10^6 $_{g^{-1}}$ of soil) At different Days After Treatment (DAT)				
			3DAT	18DAT	33DAT	48DAT	63DAT
PCNB							
	RI	120.000	0.04^{d}	0.04^{e}	0.04^{e}	0.04^{e}	5.1^{g}
	RII	240.000	0.02^{e}	0.02^{f}	0.02^{f}	3.8^{e}	4.6^{g}
	RIII	360.000	0.01^{ef}	0.01^{fg}	0.01^{fg}	3.2^{d}	4.0^{g}
Benomyl							
	RI	225.0	4.41^{a}	5.25^{a}	6.46^{a}	8.00^{b}	22.11^{a}
	RII	450.0	4.01^{a}	4.48^{b}	5.81^{b}	6.77^{c}	18.86^{b}
	RIII	675.0	3.11^{b}	3.81^{c}	4.66^{c}	5.79^{c}	16.01^{c}
Captan							
	RI	50.0	0.08^{c}	0.08^{d}	2.01^{d}	6.05^{c}	19.63^{d}
	RII	100.0	0.04^{d}	0.04^{e}	01.00^{d}	04.55^{d}	08.14^{e}
	RIII	150.0	0.02^{e}	0.02^{f}	0.05^{e}	03.70^{e}	06.12^{f}
Ceresan							
	RI	25.0	0.00^{f}	0.00^{g}	0.00^{g}	0.04^{e}	04.15^{g}
	RII	50.0	0.00^{f}	0.00^{g}	0.00^{g}	0.02^{f}	02.03^{g}
	RIII	75.0	0.00^{f}	0.00^{g}	0.00^{g}	0.01^{f}	0.08^{h}
Control (water)		0.0	4.60^{a}	6.00^{a}	8.98^{a}	10.15^{a}	18.39^{b}

RI =Rate of application below the Recommended Rate; RII = Recommended Rate of Application; RIII = Rate of Application above Recommended Rate.
Value with different alphabets in the same column are significantly different ($p \geq 0.05$) Duncan Multiple Rage Test

Table 2: Effect of different rates of fungicides application on actinomycetes populations of soil at different days after treatment

Rate of application ($\mu g\ g^{-1}$)			Mean population (cfu×10^5 $_{g^{-1}}$) At different Days after Treatment (DAT) 3DAT	18DAT	33DAT	48DAT	63DAT
PCNB							
	RI	120.000	0.05^{e}	0.05^{e}	0.05^{d}	0.05^{e}	0.05^{e}
	RII	240.000	0.03^{e}	0.03^{e}	0.03^{d}	0.03^{c}	0.03^{e}
	RIII	360.000	0.01^{e}	0.01	0.01^{d}	0.01^{d}	0.01^{e}
Benomyl							
	RI	225.0	7.60^{a}	7.81^{a}	10.22^{a}	13.81^{a}	16.03^{a}
	RII	450.0	3.60^{a}	7.81^{a}	10.22^{a}	13.81^{a}	16.03^{a}
	RIII	675.0	3.60^{b}	7.81^{a}	10.22^{a}	13.81^{a}	16.03^{a}
Captan							
	RI	50.0	3.30^{b}	4.35^{b}	8.00^{b}	9.08^{b}	14.28^{b}
	RII	100.0	3.21^{b}	3.80^{c}	7.95^{b}	8.21^{b}	13.60^{b}
	RIII	150.0	3.00^{e}	3.36^{c}	6.98^{b}	7.99^{b}	13.57^{b}
Ceresan							
	RI	25.0	01.43^{d}	01.43^{d}	01.43^{c}	01.43^{c}	17.81^{c}
	RII	50.0	0.09^{e}	00.09^{e}	00.09^{e}	00.09^{e}	8.22^{c}
	RIII	75.0	3.40^{b}	00.04^{e}	00.04^{d}	00.04^{c}	5.10^{d}
Control (untreated)		0.0	3.40^{b}	5.25^{b}	7.98	9.01^{b}	13.55^{b}

PCNB =Pentachloronitrobenzene; RI = Rate of Application below the Recommended Rate; RII = Recommended Rate of Application; RIII = rate of Application above the Recommended rate. Values with Different alphabets in the same column are significantly different (p = 0.05) Duncan Multiple Range Test

Table 3: Effect of different rates of fungicides application on fungi populations in the soil at different days after treatments

Rate of Application ($\mu g\ g^{-1}$)			Mean population (cfu×10^4 $_{g^{-1}}$) At different Days After Treatment (DAT) 3DAT	18DAT	33DAT	48DAT	63DAT
PCNB							
	RI	120.000	0.06^{d}	0.06^{d}	0.06^{d}	0.06^{d}	05.08^{e}
	RII	240.000	0.04^{d}	0.04^{d}	0.04^{d}	0.04^{d}	03.15^{f}
	RIII	360.000	0.00^{d}	0.00^{d}	0.00^{e}	0.00^{e}	02.58^{ef}
Benomyl							
	RI	225.0	6.01^{a}	8.06^{a}	10.25^{a}	13.01^{a}	15.38^{a}
	RII	450.0	4.81^{b}	6.52^{b}	08.77^{b}	10.99^{b}	12.38^{a}
	RIII	675.0	2.99^{c}	3.51^{c}	05.68^{c}	07.52^{c}	10.01^{c}
Captan							
	RI	50.0	01.01^{d}	01.01^{d}	01.01^{d}	01.01^{d}	07.03^{d}
	RII	100.0	00.06^{d}	00.06^{d}	00.06^{d}	00.06^{d}	05.05^{e}
	RIII	150.0	00.02^{d}	00.02^{d}	00.02^{de}	00.02^{d}	02.99^{f}
Ceresan							
	RI	25.0	0.00^{d}	0.06^{d}	0.06^{d}	0.00^{e}	03.01^{ef}
	RII	50.0	0.00^{d}	0.04^{d}	0.04^{e}	0.04^{e}	02.99^{ef}
	RIII	75.0	0.00^{d}	0.00^{d}	0.00^{e}	0.00^{e}	01.11^{ef}
Control (untreated)		0.0	3.20^{c}	3.80^{c}	5.87^{c}	7.91^{c}	10.23^{c}

PCNB =Pentachloronitrobenzene; RI = Rate of Application below the Recommended Rate; RII = Recommended Rate of Application; RIII = Rate of Application above the Recommended Rates. Values with Different alphabets in the same column are significantly different (p = 0.05) Duncan Multiple Range Test

bacteria population of 22.11×110^6 cfu g^{-1} of soil was observed in the soil sample treated with benomyl at lowest rate of 225.0($\mu g\ g^{-1}$) and 63 DAT when compared with that of untreated soil sample (Table 1). Bacteria right from 48 DAT recolonized soil sample treated with ceresan.

The effect of different rates of fungicides application on the population of actnomycetes in the soil at different days after treatment is presented in Table 2. It is clearly showed that the soil treated with PCNB at the lowest concentration of 36,000 $\mu g\ g^{-1}$ gave significantly lowest ($p = 0.05$) actinomycetes population of 0.03×10^5 cfu g^{-1} compared to all other treatments throughout the period of treatment (Table 2). The significantly highest ($p = 0.05$) actinomycetes population of 16.03×10^5 cfu g^{-1} was recorded in the benomyl treated soil even at the lowest rates of application and at 63 DAT (Table 2). The actinomycetes population in the captan and ceresan treated soil samples increases with days after treatment (Table 2).

In addition, the ceresan at lowest concentration of 25 $\mu g\ g^{-1}$ and PCBN at highest concentration of 360,000 $\mu g\ g^{-1}$ completely inhibited the fungi population respectively until 48 DAT but started recolonizing the soil right from 63 DAT (Table 3). The significantly highest ($p = 0.05$) fungal population (15.38×10^4 g^{-1}) noticed in the soil treated with the lowest concentration of benomyl compared to that of other treatments including the untreated soil.

Table 4: Effect of different rates of fungicides application on protozoan populations in the soil at different days after treatments

Rate of Application ($\mu g\ g^{-1}$)			Mean protozoan population ($cfu \times 10^1\ g^{-1}$) At different Days After Treatment (DAT)				
			3DAT	18DAT	33DAT	48DAT	63DAT
PCNB							
	RI	120.00	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}	0.00^{f}
	RII	240.00	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}	0.00^{f}
	RIII	360.00	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}	0.00^{f}
Benomyl							
	RI	225.0	1.5^{b}	1.50^{b}	1.50^{b}	3.58^{b}	5.11^{b}
	RII	450.0	1.3^{c}	1.30^{c}	1.30^{c}	2.69^{c}	3.95^{c}
	RIII	675.0	1.0^{d}	1.0^{d}	1.0^{d}	1.91^{d}	2.10^{d}
Captan							
	RI	50.0	0.05^{e}	0.05^{e}	0.05^{e}	0.05^{e}	01.02^{e}
	RII	100.0	0.02e	0.02^{e}	0.02^{e}	0.02^{e}	0.05^{ef}
	RIII	150.0	0.01^{e}	0.01^{e}	0.01^{e}	0.01^{e}	0.08^{e}
Ceresan							
	RI	25.0	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}
	RII	50.0	0.00^{d}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}
	RIII	75.0	0.00^{d}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{f}
Control (untreated)		0.0	2.23^{a}	2.81^{a}	3.92^{c}	4.88^{a}	6.38^{a}

PCNB =Pentachloronitrobenzene; RI = Rate of Application below the Recommended Rate; RII = Recommended Rate of Application; RIII = Rate of Application above the Recommended Rates. Values with Different alphabets in the same column are significantly different ($p = 0.05$) Duncan Multiple Range Test

The result on the effect of different rates of fungicides application on protozoan population in the soil at different days after treatment is presented in Table 4. The results revealed that PCBN and ceresan at all rates of application completely inhibit the protozoan throughout the period of treatments (Table 4). In addition, the captan treated soil significantly reduced ($p = 0.05$) the protozoan population at all tested rates of application, compared to that of the untreated soil sample (96.38×101 cfv g^{-1}) which gave the highest protozoan population (Table 4).

DISCUSSION

The present study conclusively revealed that fungicides used exhibited differential effects on microbial population of soil. The result showed that Pentachloro Nitrobenzene (PCNB) appeared to be very potent biocidal compound as it completely eliminated all the protozoan and fungal propagules in the treated soils and depressed the population of bacteria and actinomycetes by about 99%. This observation is similar to the findings of Ekundayo (2003), Odeyemi (2005) who observed that PCNB completely suppressed the growth of protozoan and fungi in forest soils of Nigeria. Furthermore, (Johnen and Drew, 1977) also reported that PNCB completely suppressed the growth of Penicillum paxilli Bair. In a related development, Curley and Burton (2005) reported that 80% of the (Rhizobium Japonican Kirchener) beitrage cells applied to PCNB treated soybean (Glycine max L. Merr.) seeds were killed by the fungicides four hours after the inoculation of the organism on the treated seeds. The relatively high toxicity of PNCB and ceresan to micro organisms might be due to the presence of five atoms of chlorine on its molecule since chlorine is potently germicidal as proposed by Cremyl (2006). The anti fungal chemical is thought to inhibit microorganisms, especially fungi by interfering with chitin synthesis (Ekundayo, 2003; Cremly, 2006).

Benomyl was observed to be hardly toxic to the soil microorganisms. It only slightly depressed the population of bacteria as well as protozoan but had no adverse effect on the population of actinomycetes. Ekundayo (2003), Vyas (2003), Cremyl (2006) reported that benomyl a wide spectrum systemic fungicides which belong to the class of benzionidazole is active against many pathogenic fungi but inactive against the phycomycetes group of fungi.

The toxicity of ceresan fungicide to all groups of soil micro organisms studied even at very low rates of application is an important finding This result corroborates the findings of Ekundayo (2003) who reported the toxicity effect of ceresan on microbial population of Mid-Western, Nigerian soil. Earlier, (Odeyemi and Ogunledun, 1983) had pointed out the inability of a cowpea rhizobium to multiply in the presence of 0.3 $\mu g\ mL^{-1}$ of cerasan. This mercurial compound affects the respiration by poisoning essential sulphydryl respiratory enzymes in bacterial and fungal cells (Vyas, 2003; Cremyl, 2006).

CONCLUSION

It is clearly showed in this study that protozoan and fungi proved more susceptible to all fungicides tested at different rates of application than bacteria land actinomycetes. Of the four fungicides investigated, pentachloronitrobenzene and ceresan were particularly toxic to soil microorganisms while benomyl was found to be least harmful even at the higher rates above that of recommended rate. The above report therefore reveals that special attention must be paid to non-target effect of fungicides application as well as usage in agricultural soils, so as to reduce their negative impact.

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