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Effect of Three Fungicides on Soil Microbial Activity and Nitrogen Dynamics

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Abstract: The objective of this investigation was to determine the effects of three fungicides on microbial activity and nitrogen dynamics of the soil. The effects of three fungicides, captan, quintozene, propamocarb hydrochloride on soil microbial activity (urease, catalase, net mineralization and soil respiration) were investigated in laboratory experiments. In each batch, incubation soil was treated with a fungicide at recommended field application rates and as a fould and incubated at 27°C for 40 days at a moisture level of field capacity. The fungicides inhibited net mineralization and nitrification at generally 4th and 8th days of incubation period. Soil respiration showed fluctuations with change in the dose and kind of the fungicide. Effect of the fungicides on catalase activity was not found statistically significant during incubation. Urease activity was significantly ($p<0.05$) affected from the fungicides.

Key words: Fungicide, soil, nitrification, mineralization, urease, catalase

INTRODUCTION

The fertility of soil, its capacity to producer crops, is a function of a range of biological processes that occur within it. The activity of the microflora of the soil is generally favourable to vegetation, e.g. the fixation of atmospheric nitrogen, the production of nitrates, sulphates and carbonic anhydride, the breaking down of plant residue and waste into compounds more easily utilised by plants and the removal from the soil of diverse products that may be added to it, such as fungicides. Biological soil system is in equilibrium, which is very sensitive to agricultural crops often expose the environment to pollution hazards. The influence of fungicides on soil microorganisms and microbial transformations in soil is dependent on physical, chemical and biochemical conditions, in addition to nature and concentration of the fungicides[1,2].

Captan applied at the recommended rates caused a depression losing in 24 h and then soil respiration increased the lag phase of the respiration rate depending upon the concentration of the fungicide. Initially, captan decreased soil respiration and the depression in carbon dioxide production was proportional to its concentration. The increase in carbon dioxide production in the later times of the experiment was probably due to the use of decomposition products of captan by microorganisms[2].

Captan, thiram and verdosan (viz., 25, 10 and 25 and 1-0 to 5.0 mg a.i. kg^{-1}, respectively) inhibited nitrification. Captan and thiram at the same rates, dicloran at 2.0 kg ha^{-1} and formalin inhibited markedly nitrification, whereas quintozene (5.6 mg a.i. kg^{-1}) had only a slight effect[2].

The applying various doses of captan into soil has showed no effect at low levels on nitrification processes, saprophyte and pathogenic fungi, however it had negative effect at high levels on biological activities[3].

Like other pesticides, fungicides are bio toxicants which interfere not only with the biochemical and physiological reactions of the target plant pathogens but may also influence populations or activity of other non-target organisms in soil[4].

The objective of this investigation was to determine the effects of three fungicides on microbial activity and nitrogen dynamics of the soil.

MATERIALS AND METHODS

This study was conducted with the soil taken from (0-20 cm depth) the research area of Agricultural Faculty of Selçuk University at 2004. Soil samples were sieved to pass 2 mm mesh and mixed homogeneously. The soil was characterized as silt loam texture and neutral pH (7.4). Organic matter and calcium carbonate contents of the soil were 2.10 and 24.30%, respectively (Table 1). The commercial grades of the fungicides, captan (N-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide, cheuron orthocide, 50% wettable powder), quintozene (Pentachloronitrobenzene, 18% wettable powder) and propamocarb hydrochloride (propyl 3 dimethlylamino, propylcarbamate hydrochloride 722 mg L^{-1}), were used in incubation experiments.

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Table 1: Some physical and chemical properties of the soil used in the study

Soil properties	Values	Soil Properties	Values
Clay (%)	18	Organic matter (%)	2.10
Silt (%)	26	$CaCO_3$ (%)	24.30
Sand (%)	56	Field Capacity (%, w/w)	17.41
Texture	Sil	Total N (mg kg^{-1} dry soil)	76.00
pH (1:2.5; soil: distilled water)	7.40	Phosphorus (mg kg^{-1} dry soil)	6.55
Electrical Conductivity (dS/m)	3.30	Potassium (mg kg^{-1} dry soil)	288.76

Incubation treatment: This study was carried out under the laboratory conditions. Three fungicides used for agricultural purposes were added into soil samples (100 g) at four levels (captan, 0, 100, 200, 400 mg a.i. kg^{-1}; quintozene 0, 36, 72, 144 mg a.i. kg^{-1} and propamocarb hydrochloride 0, 144, 288, 576 mg a.i. L^{-1}) and the soil samples were incubated at 27°C at different incubation periods (0, 2, 4, 8, 16, 32 and 40 days). Ammonia sulphate (100 ppm N) was given into each pot as basic fertilizer. In addition, the soil samples were watered at 70% of the field capacity and the soil water content was kept steady during the incubation period. The soil respiration (amount of CO_2 evolved), urease, catalase, net nitrification and mineralization values were measured at during of the period by regulating intervals.

Analysis methods: Some physical, chemical and biological properties of the soil sample were determined as follows, grain size distribution by Bouyoucos[5], pH in 1:2.5 (w/v) soil water suspension by pH meter, Electrical Conductivity (EC) in the same soil suspension by EC meter[6], organic material by using modified Walkley-Black Method[7], total nitrogen and NO_3-N using the Kjeldahl method as specified by Bremner[8], lime content by Scheibler calcimeter[9], available potassium in 1:5 (w/v) soil/water suspension[10], field capacity[11], soil urease activity by the buffered method reported by Kandeler and Gerber[12]. Carbon dioxide evolution was determined in barit solution trapped as $BaCO_3$ and found remaining solution quantity as titrimetric[13] and catalase activity with gasometric method reported by Beck[14]. Net mineralization was calculated as the difference the soil inorganic N ($NH_4^-N + NO_3^-N$) concentrations between two sampling dates. Net nitrification was also calculated as the difference from the soil NO_3^-N concentration between two sampling dates[15].

The analysis of variance procedure[16] was carried out to compare the effects of biological and nitrogen mean separations were conducted using Least Significant Differences (LSD) at $p<0.05$ tests when ANOVA indicated a significant F-value[17].

RESULTS

Effects on biological activity: There was an increasing microbial activity (urease, catalase and soil respiration) in all of fungicide treated at the beginning of the experiment (Table 2-4). Captan had temporarily more effect on soil respiration than propamocarb hydrochloride and quintozene in early incubation. Then the amount of CO_2 evolved inclined to decline in high levels of fungicides during the first 8 days of the incubation, but it increased gradually to after 40 days in the fungicide treated soil. The effect of fungicides kind x level interaction on soil respiration was important statistically ($p< 0.05$) at all the incubation period. The highest values were determined with the propamocarb hydrochloride (dose 2) and quintozene (dose 4) at the 16th day of incubation. At the same time, the highest values applying of captan was also determined at the end of incubation period (Table 2-4). Furthermore, it was found that soil respiration showed fluctuations with change in the dose and kind of fungicides.

Table 2: Effects of Propamocarb Hydrochloride (PH), Quintozene (Q) and Captan (C) on catalase activity (mg O_2 g^{-1}, mean±SE n=3)

	Dose	Incubation period (Days)						
	(mg kg^{-1})	1	2	4	8	16	32	40
Control		39.67±2.01	39.17±1.23	42.50±0.95	37.67±0.70	30.83±2.05	27.33±0.87	28.67±1.11
PH	144	44.50±0.67	40.67±0.50	38.67±0.17	38.83±1.04	32.50±0.50	29.50±1.96	28.17±1.74
	288	40.00±2.03	37.33±1.17	37.83±1.04	36.00±0.67	28.67±2.17	28.17±0.17	27.50±1.45
	576	41.33±1.45	40.50±1.64	37.17±1.74	37.00±0.60	31.50±1.45	27.67±0.44	29.17±1.92
Q	36	42.33±1.76	35.83±1.36	38.67±1.42	37.83±1.76	30.67±0.93	26.33±0.58	29.83±0.60
	72	40.67±0.88	38.17±1.04	37.33±0.73	39.50±1.45	29.17±1.48	27.00±2.65	27.67±1.15
	144	40.33±2.03	36.00±1.17	36.33±1.04	39.33±0.76	27.17±2.17	30.00±0.17	29.00±1.45
C	100	43.67±1.86	36.33±0.33	39.00±0.58	37.33±1.01	33.50±2.25	27.67±0.33	30.67±1.42
	200	41.00±1.00	37.83±1.59	37.33±1.76	37.17±0.44	30.67±0.83	27.17±1.09	27.83±1.36
	400	38.67±1.76	37.33±1.20	37.50±1.32	37.33±0.73	26.83±0.88	25.17±1.42	29.67±1.64

Table 3: Effects of Propamocarb Hydrochloride (PH), Quintozene (Q) and Captan (C) on soil respiration (mg CO_2 g^{-1} , mean ± SE n=3)

	Dose	Incubation period (Days)					
	(mg kg^{-1})	2	4	8	16	32	40
Control		16.57±6.28	9.31±3.15	8.97±1.88^{b}	2.76±2.20^{e}	9.69±3.12	27.69±8.92
PH	144	19.31±5.92	8.96±0.69	4.12±11.1^{b}	42.22±0.00^{a}	11.77±6.00	17.30±0.69
	288	13.08±6.60	8.27±3.08	2.39±2.77^{b}	18.34±1.20^{bcde}	6.58±6.35	15.23±11.0
	576	16.55±1.83	14.50±3.17	29.04±1.00^{a}	21.45±3.00^{bcd}	11.77±1.93	22.84±3.17
Q	36	14.47±3.66	11.73±4.99	1.35±0.69^{b}	7.95±7.02^{de}	6.23±0.90	29.76±1.20
	72	22.78±6.16	14.51±4.99	2.74±4.85^{b}	13.84±10.7^{cde}	13.33±2.75	31.84±12.5
	144	9.62±6.60	13.12±3.08	15.20±2.77^{b}	27.68±1.20^{abc}	6.57±6.35	40.14±11.0
C	100	22.78±10.7	4.12±0.69	12.42±0.69^{b}	31.14±5.67^{ab}	5.88±0.69	37.37±8.16
	200	26.84±9.89	9.66±3.17	6.89±5.54^{b}	30.45±8.51^{abc}	29.76±7.00	25.95±1.25
	400	12.05±3.00	17.27±13.8	6.20±1.83^{b}	22.17±3.01^{bcd}	33.23±1.73	23.53±1.20

Table 4: Effects of Propamocarb Hydrochloride (PH), Quintozene (Q) and Captan (C) on urease activity (mg N100 g^{-1}, mean ± SE n=3)

	Dose	Incubation period (Days)						
	(mg kg^{-1})	1	2	4	8	16	32	40
Control		21.77±0.57^{ab}	12.93±1.79^{bc}	20.86±2.09	2.72±0.53^{de}	8.84±1.35^{c}	2.78±0.45^{de}	4.08±0.87^{b-d}
PH	144	21.54±0.26^{ab}	21.31±1.31^{a}	10.43±4.45	5.44±1.31^{b-d}	20.63±0.92^{a}	9.52±0.13^{a}	3.40±0.00^{e}
	288	21.32±0.26^{ab}	21.76±1.97^{a}	5.44±5.76	6.77±0.79^{ab}	9.75±0.39^{c}	2.27±0.30^{de}	7.48±0.79^{a}
	576	18.14±1.83^{b}	19.04±1.57^{a}	18.14±3.01	6.35±2.23^{a-c}	8.84±0.92^{c}	5.21±1.83^{bc}	5.89±0.79^{a-c}
Q	36	25.40±3.01^{a}	17.23±0.00^{ab}	14.74±4.32	6.12±0.10^{a-c}	14.74±0.53^{b}	5.90±0.13^{b}	2.72±1.70^{de}
	72	25.17±0.92^{a}	18.14±1.05^{a}	18.36±0.92	8.34±0.00^{a}	7.25±1.44^{c}	8.84±1.05^{a}	5.21±0.92^{a-d}
	144	18.36±0.26^{b}	21.77±1.97^{a}	10.66±5.76	8.16±0.79^{ab}	7.03±0.39^{c}	4.08±0.30^{b-d}	6.57±0.79^{ab}
C	100	9.07±2.62^{c}	8.62±1.83^{cd}	3.49±0.34	3.63±0.53^{c-e}	1.58±0.39^{d}	3.62±0.00^{cd}	2.06±0.91^{e}
	200	4.31±0.66^{d}	4.99±0.26^{d}	2.26±0.00	0.90±0.00^{e}	2.49±0.65^{d}	0.90±0.00^{e}	2.26±0.00^{e}
	400	4.53±0.26^{d}	4.76±0.92^{d}	4.31±0.92	0.90±0.00^{e}	3.17±0.00^{d}	1.36±0.00^{e}	2.04±0.39^{e}

Table 5: Effects of Propamocarb Hydrochloride (PH), Quintozene (Q) and Captan (C) on net mineralization (mg N kg^{-1} d^{-1}, mean ± SE n=3)

	Dose	Incubation period (Days)						
	(mg kg^{-1})	1	2	4	8	16	32	40
Control		257.84±07.33^{bc}	28.53±08.88^{ab}	-2.23±7.33^{c}	-3.36±2.38	24.80±0.63	3.82±1.08	4.33±2.59^{a-c}
PH	144	283.89±04.11^{ab}	-1.14±06.770^{b}	8.77±9.26^{bc}	-7.23±3.63^{c-d}	28.31±0.68^{ab}	3.72±0.65	1.63±1.51^{bc}
	288	258.39±12.30^{bc}	36.48±16.40^{ab}	-7.30±6.35^{c}	6.84±1.69^{ab}	22.79±0.59^{cd}	2.89±0.66	9.37±2.17^{a}
	576	300.37±12.00^{a}	-55.52±01.80^{c}	16.41±2.60^{b}	10.49±1.91^{a}	23.34±1.10^{b-d}	2.90±0.78	6.50±0.83^{ab}
Q	36	237.43±02.35^{c}	40.31±04.75^{a}	-0.28±7.16^{bc}	4.00±3.26^{a-c}	19.33±2.38^{de}	6.47±1.54	-0.43±1.65^{bc}
	72	266.67±08.20^{a-c}	17.31±06.45^{ab}	-2.19±2.73^{c}	-9.81±1.54^{d}	31.90±1.76^{a}	5.40±0.72	4.1±0.39^{a-c}
	144	297.19±08.96^{a}	26.57±12.20^{ab}	-9.28±6.07^{c}	-15.14±5.99^{de}	22.94±1.17^{cd}	5.51±0.37	1.39±1.19^{bc}
C	100	273.68±05.78^{ab}	-69.19±02.50^{c}	40.78±4.09^{a}	-13.39±3.53^{de}	18.34±1.21^{de}	6.13±0.61	-1.41±1.39^{c}
	200	285.60±06.53^{ab}	-54.69±02.65^{c}	43.62±4.36^{a}	-24.52±3.93^{e}	12.80±2.14^{f}	8.81±2.74	-7.78±2.77^{d}
	400	188.83±07.45^{d}	43.74±15.90^{a}	36.17±3.61^{a}	-25.78±5.20^{e}	15.67±1.76^{ef}	2.66±0.42	0.04±0.70^{c}

Table 6: Effects of Propamocarb Hydrochloride (PH), Quintozene (Q) and Captan (C) on net nitrification (mg N $kg^{-1}d^{-1}$, mean ± SE n=3)

	Dose	Incubation period (Days)						
	(mg kg^{-1})	1	2	4	8	16	32	40
Control		108.19±03.07^{cd}	47.38±04.30^{a}	2.48±05.33^{bc}	4.67±2.00^{ab}	29.26±0.48^{bc}	8.09±0.94^{b-d}	3.64±2.47^{ab}
PH	144	105.34±02.65^{cd}	36.46±00.01^{a}	12.20±07.19^{a-c}	-2.10±3.21^{bc}	32.18±0.51^{b}	8.21±0.65^{b-d}	0.79±1.25^{b}
	288	132.78±02.66^{a-c}	32.08±07.94^{a}	-5.36±06.94^{cd}	7.25±2.18^{a}	28.44±0.40^{c}	7.16±0.48^{cd}	8.41±2.16^{a}
	576	137.01±10.6^{ab}	-2.68±08.23^{ab}	14.05±04.10^{ab}	8.87±1.48	29.74±0.26^{bc}	6.50±0.57^{d}	6.04±0.80^{ab}
Q	36	77.74±02.66^{e}	63.52±04.90^{a}	3.48±02.64^{bc}	7.48±2.02^{a}	22.07±1.72^{d}	10.30±1.28^{ab}	1.30±2.04^{b}
	72	139.15±04.03^{a}	-69.73±06.72^{b}	-2.59±03.20^{b-d}	-8.49±1.27^{e}	35.59±1.57^{a}	10.45±0.70^{ab}	5.04±2.04^{ab}
	144	128.43±10.6^{a-c}	33.54±06.16^{a}	-18.85±11.19^{d}	-0.46±4.93^{ab}	26.06±0.89^{c}	8.87±0.56^{b-d}	1.72±1.12^{b}
C	100	91.69±05.30^{de}	0.31±08.43^{ab}	26.39±00.40^{a}	-10.06±0.64^{e}	20.62±1.07^{d}	9.75±0.537^{a-c}	-0.25±0.98^{b}
	200	124.02±07.95^{a-c}	-24.81±10.60^{ab}	28.97±03.48^{a}	-21.03±0.77^{d}	15.50±0.52^{e}	11.93±2.39^{a}	-6.54±2.60^{c}
	400	86.40±03.84^{de}	19.26±11.10^{a}	14.67±03.82^{ab}	-19.38±0.26^{d}	15.95±0.57^{e}	3.70±0.30^{e}	1.80±0.42^{b}

Values with same letter(s) in columns are not significantly difference at $p<0.05$ according to LSD test

The effect of fungicides used in the study on catalase activity was not found significant statistically at all the incubation period. Some researchers indicated that catalase activity could be inhibited by soil properties such as pH, $CaCO_3$ and organic matter[18,19]. However, the organic matter content of soils used in this experiment is also low and $CaCO_3$ level is high amount. Whereas, effect of the fungicides on urease activity was found significant ($p<0.05$). There was an increasing urease activity in all of fungicides within first four days and then this values decreased until end of the incubation period. The highest effect on depletion of urease activity was showed by captan. The lowest effect was also obtained with propamocarb hydrochloride treatment.

Effects on nitrogen dynamics: The effects of fungicides used on ammonium oxidation or net nitrification and mineralization changed with fungicide kind, dose and incubation time (Table 5 and 6). This effect was significant statistically ($p<0.05$) at all incubation period. The applying of propamocarb hydrochloride and quintozene into soil increased ammonium oxidation at during incubation, that is, these fungicides in the soil sometimes stimulated nitrification. Whereas, the application of captan to soil decreased net nitrification, i.e. nitrification rate was inhibited by captan. The highest declining was determined with the captan application at the four days of incubation period. The application of fungicides had different effect on net nitrification and mineralization.

DISCUSSION

The influence on microbial processes and soil microorganisms of fungicides used depends on many factors. Some of major factors include physical, biochemical properties (such as pH, organic matter, temperature and moisture) of soil and nature and concentration of the fungicides applying and its time[2,20].

Fungicides applied for certain purpose on soil killed or inhibited the target and non-target microorganisms. This event could lead to an immediate inhibition of important reactions such as enzyme reactions, microbial respiration, mineralization and nitrification. The dead microorganisms or fungicides, however, might begin to serve as a substrate for the other living microorganisms. So, they are released from competition with fungi or antagonistic inhibition via substances produced by fungi. Both of these effects could lead to a rapid flush of microorganism activity would be likely to increase the rates of mineralization of nitrogen from organic materials in the soil, thereby nitrogen availability.

The source mineralization nitrogen may include the dead fungal biomass, the fungicides, or other soil organic materials. Captan, propamocarb hydrochloride and quintozene mixed into soil not only affected on fungus but also on bacteria and other microorganisms. Because of this effects of fungicides, the nitrification, denitrification, soil respiration and some enzyme activity, which are important for soil productivity, could be influenced.

In this study, it was determined that the fungicides showed prohibiting effect on important biochemical processes. Propamocarb hydrochloride, quintozene and captan decreased soil respiration primarily during the first eight days, but then it started rising toward the end of the incubation. It has been reported that soil respiration was inhibited after applying of fungicides in short time[4,21], but reductions were only temporary and populations of surviving microorganisms recovered soon and the respiration rate often rose to relatively high levels[22]. This was probably based not only on the recovery of the original microbial population but also on the increased populations and activity of a few resistant microbial species, or was due to direct microbial utilization of fungicides as substrates[23].

Concentrations of total inorganic N (NH_4^-N and NO_3^-N, data not given) were significantly increased by the three fungicides, possibly due to higher rates of mineralization, which resulted in higher NH_4-N concentration in soils. The highest NH_4-N concentrations released in captan treated soils and higher net mineralization and nitrification rates probably resulted from the mineralization of dead organisms at initial incubation period. The other researchers reported that applying of fungicides at normal rates retarded nitrification significantly[24,25]. The reductions in NO_3^-N and increases in NH_4^-N concentrations as reported in this study were probably due to the nitrifying bacteria being susceptible to captan[2,26] or the altering the ratio and numbers of fungi and heterotrophic bacteria in soils[27,28]. Propamocarb and quintozene enhanced net mineralization and nitrification but captan reduced nitrification rate. In our investigation, it was able to confirm some short-term (less than 30 days or more) side effects of the three fungicides tested on soil microbial activity and nitrogen dynamics. These effects were temporarily but the results indicated that the overall impact of fungicides on soil ecosystems depended not only on the different type levels and mode of action of fungicides.

Tested fungicides had generally some inhibitory effects on soil microbial activity (urease and soil respiration), but catalase activity has been slightly affected by fungicide treatment. It was clear, from present findings and other reports[9,15,16], that these fungicides were inhibitory as well as stimulators certain groups of microorganisms in the soil. The each fungicide exhibited quite different effects on the soil process. Quintozene and captan in comparison with propamocarb appeared to have worn profound effects on overall nitrogen dynamics, although none of the effects of the three fungicides on soil microbial activity and nitrogen dynamics were large or long-lived. The results lead to the need for monitoring such effects for commonly used fungicides.

In conclusion, the further researches require the trials with soils having different organic matter, texture and pH and with more fungicides type, dose and incubation period under laboratory, greenhouse and field conditions.

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