AN EVALUATION OF FUNGICIDES AS NITRIFICATION INHIBITORS

By

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C E R T I F I C A T E I

This is to certify that this thesis entitled "An evaluation of fungicides as Nitrification Inhibitors" submitted for the degree of M.Sc. in the subject of Microbiology of the Haryana Agricultural University, Hisar, is a bonafide research work carried out by Miss Mamta Rani under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Shashi Pasrda.
Major Advisor

CERTIFICATE - II

This is to certify that the thesis entitled, "An Evaluation of Fungicides as Nitrification Inhibitors" submitted by Miss Mamta Rani to the Haryana Agricultural University, Hisar in partial fulfilment of the requirements for the degree of M.Sc. in the subject of Microbiology has been approved by the student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

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CHAPTER - I

INTRODUCTION

Urea constitutes the major nitrogen fertilizer at present and its use is steadily increasing in the world agriculture. It contributes to about 80% of the total fertilizer-N. In most soils, urea is rapidly hydrolized to ammonium carbonate through urease and results in the formation of ammonium which is subsequently nitrified to nitrate nitrogen.

$$NH_2CO.NH_2 + H_2O \longrightarrow (NH_4)_2CO_3 \longrightarrow 2NH_4^+ + CO_3^{-2}$$

The ammonium formed in the soil is converted to nitrate *via nitrite through nitrification process* i.e. the biological oxidation of ammonium to nitrate via nitrite mediated by Nitrosomonas and Nitrobacter species of nitrifying bacteria respectively. Through nitrification, the slowly leached and relatively immobile NH_4^+ cation which is bound to cation exchange complex of organic matter and clay particles, is converted to NO_3^- anion. Nitrate ions are most readily assimilated by most of the crops through plant roots but it is not so well retained in the soil as compared to ammonical nitrogen. Nitrate is susceptible to losses through leaching and denitrification in soils. The formation of nitrogen gases as a result of denitrification

not only leads to the loss of nitrogen but creates problems of atmospheric pollution which includes pollution of both air and water. Leaching is prominent in light textured soils during heavy rainfall or intensive irrigation while denitrification may be predominant in water logged and heavy soils.

Quantitative estimates of nitrogen losses from agricultural soils vary on an average from 20-70% (Prasad et al., 1971; Craswell, 1978). The losses are relatively less in arable soils as compared to water logged soils which are more prone to denitrification reactions.

Several approaches have been followed to decrease the losses of fertilizer nitrogen and to increase the efficiency of nitrogen uptake and use by the plants. These include development of slow release fertilizers, specific inhibitors of micro-organisms and use of relatively insoluble coatings on fertilizer granules. The use of slow release fertilizers has either not been popular either because their mineralization is slow or the production cost is high (Hauck, 1972; Trivedi and Pachaiyappan, 1979). Sulphur coated urea, although effective, has not been used in large extent because of high cost of sulphur. Similarly neem cake or its extract although effective as a coating agent, is only available in limited quantity. Recently

the emphasis has been more on nitrification inhibitors because these may be effective at a very low concentrations.

The materials developed or adopted specifically as nitrification inhibitors include N-Serve or nitrapyin (2-chloro-6-(trichloromethyl) pyridine), AM (2-amino-4-chloro-6-methyl pyrimidine), ST (2-sulfanilamido thiazole), terrazole (5-ethoxy-3-trichloro methyl-1, 2, 4, thiadizole), KN_3 (Potassium azide) and dicyanadiamide.

Much research work has been done on the effect of pesticides on different soil microbiological processes and most of these chemicals do not adversely affect these processes at the concentration in which they are recommended for use. However, amongst all the soil biological processes, nitrification is most sensitive to pesticides (Alexander, 1978; Anderson, 1978) and there has been a trend in recent years to assess the possibility of using pesticides as nitrification inhibitors because of the fact that pesticides are commercially available and most of them do not have prohibitive costs. During the present investigations two fungicides zineb and captafol were selected for assessing their use as nitrification inhibitors since these and other related fungicides have earlier been reported to inhibit nitrification in soil at less than 20 µg/g concentration (Hill and Wright, 1978). The following

studies were conducted:

1. Growth studies of Nitrosomonas and Nitrobacter to determine the growth pattern and optimum substrate concentration.

2. Effect of two fungicides (zineb and captafol) on Nitrosomonas and Nitrobacter in liquid culture.

3. Effect of fungicides on nitrification of $(NH_4)_2SO_4$ and mineralization of urea in soil.

CHAPTER - II

REVIEW OF LITERATURE

The decomposition of proteins, nucleic acids and other nitrogenous organic substances in soil leads to the release of NH_3 which equilibrates to the ionic species, NH_4^+ in almost all soils. Ammonium may also be added in the soil by the hydrolysis of urea or through ammoniacal fertilizers. In most of the soil conditions, NH_4^+ is oxidized to NO_3^- as rapidly as it is formed. The process whereby NH_4^+ is oxidized to NO_3^-, is referred to as nitrification. Nitrification takes place in almost all soils where NH_4^+ is present and the conditions are favourable with respect to major factors of temperature, moisture, aeration and pH.

It occurs in terrestrial, aquatic and sedimentary ecosystems. The strictly biological nature of nitrification was firmly established with the isolation of "nitrifying bacteria" by Winogradsky in the period of 1889-1890. He described representatives of two small groups of specialized chemoautotrophs and clearly related the metabolism of each of the two corresponding stages of nitrification: the oxidation of NH_4^+ to NO_2^- and then oxidation of NO_2^- to NO_3^- . The autotrophic nitrifying bacteria are the principal agents of

nitrification in soil and other ecosystems.

Nitrifying organisms:

Nitrification is largely brought about by chemoautotrophic bacteria, although it has been established that some heterotrophic organisms are capable of producing NO_2^- and NO_3^- from NH_4^+ and organic nitrogen in pure cultures (Eylar and Schmidt, 1959; Gunner, 1963; Doxtader and Alexander, 1966; Focht and Verstraete, 1977). This form of nitrification has been observed only in pure cultures and rarely in soils.

Autrotrophic nitrifiers: Gram negative chemoautotrophic nitrifying bacteria comparising the family Nitrobacteriaceae are directly linked to nitrification in natural environments. List of chemoautotrophic nitrifiers expanded from Bergey's Manual of Determinative Bacteriology, 8th Ed. (Watson, 1974) is as follows:

Table 1

List of chemoautotrophic nitrifying bacteria(Watson,1974)

Genus	Species	Habitat
Oxidize $NH_4^+ \rightarrow NO_2^-$		
Nitrosomonas	europaea	Soil,water,sewage
Nitrosospira	briensis	soil
Nitrosococcus	nitrosus	Marine, soil
	oceanus	Marine
	mobilis	Marine
Nitrosolobus	multiformis	Soil
Nitrosovibrio	tennis	Soil
Oxidize $NO_2^- \rightarrow NO_3^-$		
Nitrobacter	winogradskyi	Soil
Nitrospira	gracilis	Marine
Nitrococcus	mobilis	Marine

NH_4^+ Oxidizing Bacteria: All genera of NH_4^+ oxidizers listed in Table 1 indlude species isolated from soil. Despite the fact that various genera could be responsible for NH_4^+ oxidation in soil, Nitrosomonas has been widely accepted to contribute all such oxidations. Nitrosomonas is the most common NH_4^+ oxidizer in soil and hence the

others are designated as "Secondary genera" (Focht and Verstraete, 1977). Nearly all physiological and biochemical studies have focussed on N. europaea, and relatively little is known about the biological features of other genera. However, all genera of NH_4^+ oxidizers appear to be basically similar with respect to energetics, carbon fixation and independence of pre-fixed organic compounds.

Nitrosomonas oxidizes NH_4^+ to NO_2^- according to the equation.

$$NH_4^+ + 1\frac{1}{2}\ O_2 \xrightarrow{6\ e^-} NO_2^- + H_2O + 2H^+;\ F\text{-}65\ Kcal.$$

This is energy yielding reaction and this energy is provided for biosynthesis and maintenance.

Nitrite oxidizing bacteria: Since only Nitrobacter is associated with soil habitat the generic diversity of NO_2^- oxidizers is less than that of the NH_4^+ oxidizers. Two species of Nitrobacter - N. winogradkyi and N. agilis were generally accepted as comprising the genus prior to 1974. In Bergey's Manual, Watson (1974) considered these two morphologically similar forms into one species, N. winogradskyi.

The oxidation of NO_2^- to NO_3^- by Nitrobacter is as follows (Nicholas, 1978).

$$NO_2^- + 1/2\ O_2 \xrightarrow{2\ e^-} NO_3^-;\ F\text{-}17.8\ Kcal$$

Nitrification by heterotrophs: The first instance of NO_3^- formation by a heterotroph was reported by Schmidt (1954) with isolation of a soil fungus, Aspergillus flavus. Eylar and Schmidt (1959) carried out a survey of microorganisms isolated from soil to determine the incidence of heterotrophic forms capable of producing NO_2^- or NO_3^-. Heterotrophic microorganisms, bacteria and fungi can be isolated from soil and cultured under conditions that lead to the accumulation of NO_2^- or NO_3^-. There is no evidence from the relatively few studies attempted that any of the heterotrophs that nitrify in culture ever do so in nature (Schmidt, 1960). Nitrification by heterotrophs as demonstrated in pure culture are not readily extended to substrate limited conditions of the soil.

Nitrification sometimes occurs in nature under soil circumstances where autotrophic nitrifiers can not grow (Focht and Verstraete, 1977). Heterotrophic nitrification is commonly evolved to account for nitrification in such instances, but no evidence of this nitrification is there to isolate a particular heterotroph, to demonstrate its potential in pure culture and then to relate its occurrence in the natural environment.

Several methylotrophs have also been shown to oxidize NH_4^+ to NO_2^- in pure culture conditions (Hutton

and Zobell, 1953; Dalton, 1977).

Role of Nitrification and Urea hydrolysis:

Nitrification process occupies a central position in soil nitrogen transformations. It is an essential process in the ecosystem and to the plants as it provides NO_3^- to the plants under arable conditions. This process is essential except under anaerobic systems where the plants prefer NH_4^+ nitrogen, NO_3^- - N is preferred under aerobic conditions by most of the plants. Urea which is most commonly used in nitrogen fertilizers also results ultimately into the production of NO_3^- first by its hydrolysis to NH_4^+ and then further oxidation of NH_4^+ to NO_3^-.

Nitrification has three general effects on the recovery of soil and fertilizer nitrogen by crop plants (Hauck, 1972). First, it affects the nitrogen nutrition of plants, because the rates of uptake and use of the substrate and end product of nitrification - ammonium and nitrate respectively, are different. Second, it promotes movement of nitrogen because it converts relatively immobile, cationic form of nitrogen to a mobile anionic form. Third, nitrification results in the formation of oxidized forms of nitrogen that can be lost from the soil by microbial and chemical action, nitrite

and nitrate by denitrification and nitrite and chemo (non-enzymatic) denitrification.

Nitrification can also result in the reduction of nitrogen immobilization and ammonia fixation. Little nitrate is immobilized by soil heterotrophs in the presence of ammonium. But it can not be concluded from the limited field data available that reduction of these two processes will result in significant increase in fertilizer efficiency. Apart from its obvious advantages the possible disadvantages of nitrification are:

a) Promotion of nitrogen loss by bio-and chemodenitrification, leaching and run off.

b) Nitrate movement beyond the crop root zone and

c) Nitrate accumulation in ground waters.

Disadvantages of nitrification outweigh the advantages therefore, the control of nitrification should lead to increased efficiency of nitrogen use, manifested as improvement in crop growth, yield and quality.

The losses of nitrogen under field conditions may be significant and may range from 10-100% (Stephenson and Ries, 1969) although under arable conditions the efficiency of fertilizer N is normally between 40-60% and under water logged conditions 20-40% (Prasad et al., 1971; Tandon,1974; Schmidt, 1982). Many natural organic materials or synthetic

compounds may inhibit nitrification because of broad biocidal activity or by toxicity to the microbes that oxidize ammonium. These materials include root extract of many plants (Rice and Pancholy, 1973), Pesticides and specific inhibitors of nitrification.

Natural Inhibition of Nitrification:

Inhibition of growth by organic compounds of various kind is well documented for nitrifiers in pure culture (Gunderson, 1955). Many toxic compounds such as certain amino acids and nitrogenous bases are released during decomposition of organic matter, but nitrification proceeds vigorously even in sewage and manure wastes so long as major environmental factors are favourable. Nitrification may be affected by certain decomposition products of organic residues or by metabolites excreted by plants or micro-organisms in soil but it is still only suggestive. Most of the research on the matter has focussed on grassland soils and the reports are highly contradictory. Clark and Paul (1970) reviewed the various reports both for and against this hypothesis. Much of the evidence for inhibition has derived from the addition of root extracts to nitrifying cultures (Rice, 1964; Munro, 1966; Neal, 1969). Rice (1964) added the extracts to liquid medium at the rate of 5%. The presence of inhibitory substances under

such soil conditions is probable but it is unlikely that similar concentrations of inhibitory substances occur in the rhizosphere. Monila and Rovira (1964) found that exudates inhibited Nitrosomonas in liquid culture, but MPN numbers of both NH_4^+ oxidizers and NO_2^- oxidizers were increased in the rhizosphere soil of the same plants.

Moore and Waid (1971) provided some of the strongest evidence for specific inhibition by plant exudates. They added fresh root washings to a soil that was adjusted to a constant nitrification rate. The most pronounced depression of nitrification was obtained with ryegrass root extracts.

Specific Inhibition of nitrification :

The use of specific nitrification inhibitors to regulate the formation of NO_3^- from ammonium fertilizer was recommended for the first time in 1962 by Goring who developed N-serve (2 chloro-6-(Trichloro methyl) pyridine) as the specific nitrification inhibitor (Goring 1962a,b). This compound is one of the best nitrification inhibitor available today and is currently registered for use in many crops in USA. This reduces nitrogen losses from leaching and denitrification at very low rates (0.05 to 20 µg/g depending upon soil types). Bremner et al. (1978), however, recommended

some precautions in the use of nitrapyrin, otherwise the chemical becomes ineffective.

Many materials will inhibit nitrification either because of broad biocidal activity or by toxicity to specific microbes that oxidize ammonium. Inhibitors affect the autotrophic nitrifiers by retarding either their growth or their functions. Inhibition of nitrifying activity can be caused by interfering with respiration and cytochrome oxidase function by chelating essential metal ions by production of acid in the micro-environment and by liberation of toxic compounds such as mercaptans, sulfoxides and sulfones.

Some of the other materials developed and recommended for this purpose include AM (2-amino, 4-Chloro-6-methyl pyrimidine), ST (2-sulfanilamideothiazole), terrazole (5-ethoxy-3-trichloromethyl-1, 2, 4-thiadizole) KN_3 (Potassium azide), didin and ammonium thiosulphate (Alexander, 1965; Huber and Watson, 1974; Prasad et al., 1971; Amberger, 1982).

The most comprehensive comparative testing of nitrification inhibitors was reported by Bundy and Bremner (1973), who used 24 compounds applied to three soils at the rate of 10 µg/g to observe effects on the nitrification

of 200 ug/g NH_4^+—N. Ten compounds had no effect on NO_3^- generation, the others were ranked as follows on the basis of decreasing average effectiveness: 2-Chloro-6-(trichloromethyl) pyridine (Nitrapyrin), 4-amino-1, 2, 4-triazole (ATC), potassium or sodium azide, 2-4 diamino-6-trichloromethyl-s-triazine (CL-1580), dicyanadiamide, 3-chloroacetanilide 1-amino-2-thiourea, 2,5-dichloroaniline, phenylmercuric acetate, 3-mercapto-1, 2, 4-triazole or 2-amino-4-sodium diethylidithiocarbamate. The first six gave more than 50% effectiveness as averaged for the three soils.

Dicyanadiamide (Didin) has been found to be an effective nitrification regulator in soils and manures in the past few years. This chemical is effective at a concentration of 5-10 µg/g in soils and is retained in soil between 40 to 60 days (Amberger and Vilsmeier, 1979; Amberger, 1982).

Work done in India has shown that non-edible oil cakes and their constituents particularly those of neem (Azadirachta indica) and Karanja (Pongamia glabra) have the ability to retard nitrification (Reddy and Prasad, 1975; Sahrawat and Parmar, 1975; Sahrawat, 1981). However, these materials were considerably inferior to nitrapyrin, Mishra et al. (1975) found that neem seed cake inhibits

Nitrosomonas.

Bremner and Douglus (1971) for the first time recommended the use of urease inhibitors for controlling nitrogen losses. They opined that urea is rapidly hydrolysed in the soil resulting in accumulation of ammonium. The increased concentration of ammonium may lead to toxicity to germinating seedlings and gaseous loss of urea N as ammonia at higher pH. Bremner and his associates (Bremner and Douglas, 1971; Bundy and Bremner, 1973, 1974; Bremner and Mulvaney, 1978; Mulvaney and Bremner, 1981) tested a large number of benzoquinones, hydroquinones and other urease and nitrification inhibitors and reported that the methyl and halogen substituted p-benzoquinones were the most inhibitory for urease activity.

Mishra and Flaig (1979) and Mishra et al. (1980) tested a large number of anthraquinones and naphthoquinones and found 4-tert butylpyrocatechol and 4, 6-ditertbutyl pyrocatechol to be effective inhibitors of urease activity and nitrification. Recently, phenylphosphorodiamidate (PPD) has been reported to be the most effective urease inhibitor (Martens and Bremner, 1984).

The urease inhibitors in general, have utility in soils of high pH where volatilization losses may be significant or in soil of light texture where retention

of NH_4^+ is less. Deep placement of urea significantly reduces nitrogen losses (Rao and Batra, 1983).

Although a large number of chemicals have been found to be useful as nitrification inhibitors but they have found limited field applications. Most of these chemicals are not produced commercially as nitrification inhibitors and therefore their prohibitive costs have limited their use.

Effect of pesticides:

Pesticides may be natural or synthetic compounds, which are recommended for use against pests and include fungicides, herbicides, insecticides and nematicides. These chemicals find their way into the soil through soil treatment, seed treatment, foliar spray etc. Apart from the target organisms a pesticide may affect other organisms present in the soil. Voluminous data is available on the effect of various pesticides on different soil microbiological processes (Alexander, 1973; Powlson, 1975; Alexander, 1978; Anderson, 1978).

Most populations of microbial groups are not reduced or greatly affected by those pesticides that are present at low concentrations, but algae are notable exceptions, many of which are markedly inhibited by herbicides. The

insecticides alter the abundance of particular heterotrophic population when present in high concentrations, although responses vary with soil type. On the other hand, fungicides and fumigants are added to sufficiently high levels to suppress a group of microorganisms. Since many indigenous heterotrophs are as susceptible to chemical stress as are the disease incitants, dramatic population modification are commonly associated with such treatments (Ridge and Theoderou, 1972).

Compounds that are found in concentration high enough to be injurious, do not affect all the populations to same extent, one species may get suppressed appreciately, a second may suffer only modest harm while a third is entirely resistant.

Nitrification is a sensitive transformation which is suppressed at a lower concentrations as compared to other processes (Morris and Giddens, 1963).

Anderson (1978) compared the ratios of effects of pesticides on different soil processes. In his attempt to obtain an overall guide to the general effects, all stimulatory effects and no effects were added and designated as positive. Similarly all inhibitions were added together and designated as negative. The ratio of positive to

negative effects was described as effect ratio. Although the effect varies on different agroclimatic conditions, but the effect ratio given in Table 2 gives general idea about the effect of pesticides on soil microorganisms and the processes brought about by these organisms.

Table 2

Summary of pesticide effect ratios on microbial processes in soil

Parameter	Herbicides	Fungicides	Insecti-cides	Other pesticides
1. Bacterial numbers	1.20	3.50	1.30	1.00
2. Nitrification	1.40	0.54	0.82	0.32
3. Denitrification	1.82	i.d.a.*	i.d.a.	i.d.a.
4. Rhizobia and legume nodulation	0.94	1.00	0.78	i.d.a.
5. Free living N_2 fixation	1.65	i.d.a.	1.75	i.d.a.
6. Fungi and actenomycetes	1.09	0.5	1.43	0.55
7. Cellulolytic activity and organic matter degradation	1.31	i.d.a.	1.10	0.62
8. Respiratory activity	0.91	0.40	2.00	1.40
9. Ammonification	1.74	1.30	1.84	1.20

* Insufficient data available.

From the ratios obtained (Table 2) it is possible to state that on the basis of data reviewed that bacterial

numbers in soil are not generally adversely affected by any of the four groups of pesticides, whereas nitrification is more sensitive to fungicides, insecticides and other pesticides than it is to herbicides.

The effect of different groups of pesticides on nitrification are described below.

Herbicides : Nitrification is one of the most extensively studied soil processes and accumulation of data on pesticides effects, makes it very difficult to generalize on the effects of these chemicals. To date, the most inhibitory chemicals, excluding those specifically designed to inhibit nitrification, are sodium chlorate and calcium cyanamide. The former can inhibit nitrifiers for upto a year whilst the latter has been reported to almost completely eliminate nitrification (Audus, 1970).

In culture studies, Winely and San Clemente (1968) studied the effect of EPTC and Chlorpropham at rates equivalent to field rates and found that NO_2^- oxidation by Nitrobacter sp. was inhibited and the higher rates stopped the process completely. Farmer et al. (1965) found that simazine at 6 µg/g inhibited Nitrobacter agilis but not Nitrosomonas europaea. Simazine at 5, 10 and 100 µg/g was found to have no effect on pure cultures of nitrifiers (Kulinska, 1967).

In soil studies, Debona and Audus (1970) using perfusion columns observed an increasing order of inhibitory effectiveness for ten herbicides against nitrification at high concentrations. The order was: dichlobenil < paraquat < picloram < 2, 3, 6, trichlorobenzoic acid = chlorthiamid < bromoxynil < chlorflurazole < ioxynil < propanil.

Domsch and Paul (1974) tested the effect of 35 herbicides on nitrification by experimentation and mathematical modelling. They concluded that most of the herbicides had a negligible effect at normal field rates and were only effective against nitrification when the soil pH was below 7.0. oxidation of NO_2^- to NO_3^- appeared to be more sensitive than oxidation of NH_4^+ to NO_2^- .

Fungicides : Fungicides may severely supress nitrification in soil. Audus (1970) concluded that strong suppression of nitrification, followed by slow recovery is shown by the following fungicides (recovery time in days in parenthesis) nabam (60), thiram (60), ferbam (28), maneb (25) and zineb (17).

Cepatan, thiram and verdasan either stimulated or had no effect on nitrification in grass soil when applied at low concentrations. At higher rates, however, captan

(25 kg/ha), Verdasan (1.0-5.0 kg/ha) and thiram (10 and 25 kg/ha) inhibited the process (Wainwright and Pugh, 1973).

In mixed cultures of Nitrosomonas sp. and Nitrobacter sp. it was found that 20 µg/ml benomyl inhibited oxidation of NO_2^- to NO_3^- and that 200 µg/ml delayed the oxidation of NH_4^+ to NO_2^- (van Faassen, 1974).

Insecticides : The insecticides are inhibitory normally at a high concentrations. Growth of Nitrobacter agilis in aerated cultures was inhibited by chlordane, 1, 1-dichloro-2, 2-di (4-chloro phenyl) ethane and heptachlor or lindane at 10 µg/g but not by aldrin, whilst lindane, TDE and heptachlor and chlordane also partially inhibited NO_2^- oxidation (Winely and San Clemente, 1970). On the other hand, Mishra et al. (1972) found thatneither lindane nor phorate, at normal and ten times normal rates, had any adverse effect on nitrification although Nitrosomonas sp. was slightly affected. Earlier, it was shown that Nitrosomonas is more sensitive than Nitrobacter to high rates of lindane (Garretson and San Clemente, 1968).

Shin Chsiang Lin et al. (1972) using loam soil, tested the effects of eight insecticides on nitrification. Carbofuran and carbophenothion had no effect, trichlorofam inhibited at 5 and 50 kg/ha for a short period, fonofos, trichlor onate and chlorpyrifas showed persistant inhibition

at 50 kg/ha and aldicarb and propoxur at 50 kg/ha showed marked inhibition of nitrification. Kuseske et al.(1974) found that at rates upto 500 µg/ml propoxur and aldicarb were toxic to both Nitrosomonas sp. and Nitrobacter sp.

Soil Fumigants and other Pesticidal chemicals : Apart from herbicides, fungicides and insecticides, the fumigant also have inhibitory effect on nitrification at relatively lower concentrations. Under field conditions metham sodium and dazomet increased numbers of nitrifying bacteria in a loam soil but allyl alcohol and formalin inhibited nitrifiers, under less dry soil conditions (Naumann, 1970; Naumann, 1971) later showed that nitrifiers in the loam soil under green house conditions were inhibited for 38 days after treatment with dazomet and for 13 days with allyl alcohol.

The nitrification of native soil nitrogen in a sandy loam was stimulated by ethoprophos and 1,3 dichloropropene at a soil temperature of 5°C for two weeks after application whereas in the same soil, ethoprophos, N-serve, 1,3-dichloropropene and vorlex inhibited the nitrification process four weeks after application (Tu, 1973).

In comparing the effects of two fumigants and N-serve on nitrification in three soils at temperatures

of 5°, 25° and 40°C Thiagalingam and Kanehiro (1971) found methyl bromide to be a more effective inhibitor in all three soils regardless of temperature. Ethylene dibromide and N-serve were also inhibitory.

Zineb, captafol and related fungicides have been reported to adversely effect nitrification in soil at 20 kg/g or lower concentrations (Goring and Hamakar, 1972; Hill and Wright, 1978) therefore, zineb and captafol were selected to assess the feasibility of their use as nitrification inhibitors.

CHAPTER - III

MATERIALS AND METHODS

Bacterial cultures :

Pure cultures of *Nitrosomonas* sp. and *Nitrobacter* sp. used during the present investigation were earlier isolated in the Department of Microbiology, Haryana Agricultural University, Hisar. The cultures were maintained in liquid medium. Transfers were made after every fifteen days. The following medium was used for growing *Nitrosomonas* sp. (Belser and Schmidt, 1978).

$(NH_4)_2SO_4$	0.5	g
KH_2PO_4	0.2	g
$CaCl_2.2H_2O$	10	mg
$MgSO_4.7H_2O$	40	mg
Bromothymol blue	2	mg

The following medium components were added in trace amounts (1-2 mg).

$FeSO_4.7H_2O$

EDTA disodium salt

$NaMoO_4.2H_2O$

$MnCl_2$

$COCl_2.6H_2O$	
$ZnSO_4$	
$CuSO_4.7H_2O$	
Distilled water	= 1 litre
pH	7.5 - 7.8.

The medium used for Nitrobacter sp. (Belser, 1977) was as follows:

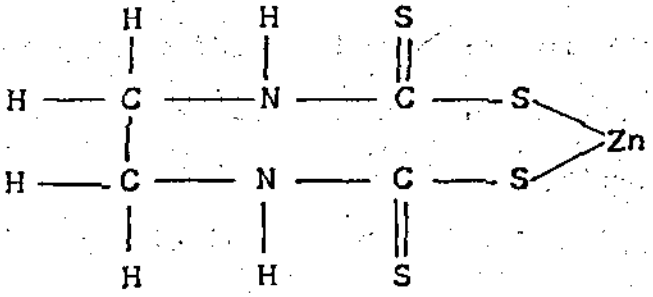
$NaNO_2$	1.0 g
Na_2CO_3	1.0 g
K_2HPO_4	0.5 g
NaCl	0.5 g
$MgSO_4.7H_2O$	0.3 g
$FeSO_4.7H_2O$	1 mg
Distilled water	1 litre
pH	8.0

Chemicals :

The chemicals used throughout the course of present investigation were obtained from commercial sources such as British Drug House, Pvt. Ltd., Bombay; Sarabhai Chemicals Ltd., Baroda, and E. Merck India Ltd., Bombay.

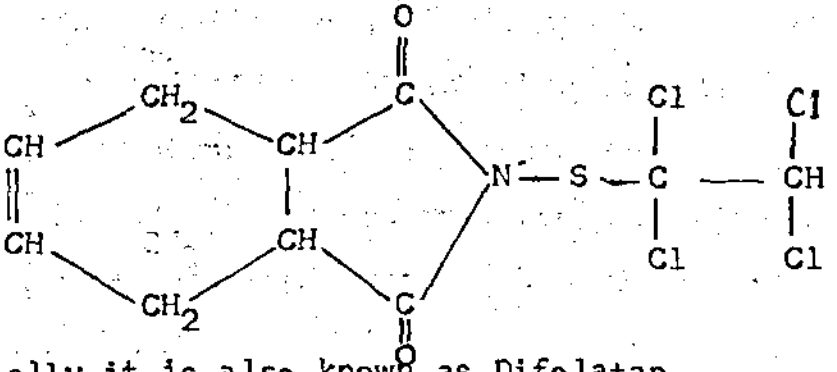
Two fungicides - zineb and captafol were commercial formulations and were obtained from Indofil Chemicals Ltd., Bombay and Rallis India Ltd., Bangalore respectively.

Zineb belongs to dithiocarbamate group. Its chemical formula is zinc ethylene bis dithiocarbamate and the chemical structure is as follows:



Commercially it is also known as Dithane Z-78.

Captafol belongs to heterocyclic nitrogenous compound. Its chemical formula is Cis-N-(1,1,2,2-tetra chloroethyl thio)-4- cyclohexane-1,2-dicarboximate and the structure is as follows:



Commercially it is also known as Difolatan.

Collection of soil samples :

Soil samples were collected from the upper 15 cm of the profile from the soils area of the Haryana Agricultural University Farm at Hisar. The soil samples were air dried, powdered and passed through a 2.0 mm sieve. The soil samples were analysed for the organic carbon, total nitrogen and pH. Organic carbon in the soil sample was estimated by the procedure of Walkley and Black (1934). Total nitrogen in the soil was estimated by microkjeldahl method as described by Bremner and Mulvaney (1982). The pH of the soil was determined in a systronics digital pH meter using a soil to water ratio of 1:2.5.

Growth pattern of Nitrosomonas sp. :

Growth pattern of Nitrosomonas sp. was studied by inoculating 1 ml of pure culture (15 days old) to 30 ml sterilized medium contained in 150 ml Erlenmeyer flasks. The flasks were incubated at 30°C on a rotary shaker. Nitrite was determined colorimeterically after every two days upto ten days (Bremner, 1965), and the most probable number count of Nitrosomonas was taken at 0 time and after 10 days of incubation by the procedure described by Schmidt and Belser (1982). The experiment was conducted in triplicate and values are average of three replications.

Determination of Nitrite Nitrogen :

Reagents :

(a) 1% Sulphanilamide: Dissolved 1 g of the sulphanilamide in 100 ml of an acid solution made up with 75 ml of distilled water and 25 ml of conc. HCl. Stored in an amber coloured reagent bottle.

(b) 0.02% N-(1-naphthyl) ethylene diamine hydrochloride: Dissolved 20 mg of the substance in 100 ml distilled water and stored in an amber coloured reagent bottle.

Procedure :

One ml of aliquot was taken in a test tube and to this was added 0.5 ml of sulphanilamide reagent followed by 0.5 ml of the naphthylene diamine reagent to develop the colour. Water was added to make the final volume to 4 ml. After 10 min. the density of the color was determined at 540 nm on a Spectronic 20 colorimeter.

Determination of MPN of Nitrosomonas :

For MPN of Nitrosomonas sp. serial dilutions were made in 0.5 mM phosphate buffer (pH 7.0).

One ml from each serial dilution was inoculated

in triplicate tubes containing 4 ml of sterilized medium. The tubes were incubated at 30°C in dark. Observations were made after 15 days by spot test.

The Spot Test was done as follows:

Reagents :

(a) 0.5 sulphanilamide in 100 ml 2.4 N HCl stored in a refrigerator.

(b) 0.3 g of N(1-naphthyl)-ethylene diamine hydrochloride in 100 ml of 0.12 N HCl stored in refrigerator.

Took 0.1 ml of aliquot aseptically in a crucible and added 1 drop of reagent (a) and 1 drop of reagent (b). Pink color indicated the presence of nitrite and was designated as positive.

Growth pattern of Nitrobacter sp. :

Growth pattern of Nitrobacter sp. was studied in the same manner as for Nitrosomonas by inoculating 1 ml of pure culture of Nitrobacter in 30 ml of sterilized NO_2^- oxidizing medium contained in 150 ml Erlenmeyer flasks and incubated at 30°C on a rotary shaker. Nitrate production was determined after every three days by salicylic acid method (Cataldo et al., 1975). The MPN count was taken

at O time and after 15 days.

Determination of Nitrate Nitrogen :

Aliquots were pipetted into 50 ml Erlenmeyer flasks and mixed thoroughly with 0.8 ml of 5% (w/v) salicyclic acid in concentrated H_2SO_4. After 20 min at room temperature 19 ml of 2N NaOH was added slowly. The flasks were then cooled to room temperature and absorbance at 410 nm was determined on Spectronic-20 colorimeter.

Determination of MPN of Nitrobacter :

For MPN of Nitrobacter sp. serial dilutions were made in phosphate buffer in the manner described for Nitrosomonas. One ml of each dilution was added to tube containing 4 ml of Nitrobacter medium and the tubes were incubated at 30°C. Spot test for the presence of nitrate was performed after 15 days. The disappearance of nitrite indicated the presence of Nitrobacter and was designated as positive. The presence of nitrite indicated the absence of Nitrobacter and was designated as negative.

Determination of Substrate Concentration for Nitrosomonas sp

Thirty ml of the Nitrosomonas medium described earlier but without ammonium sulphate was taken in 250 ml

Erlenmeyer flasks and $(NH_4)_2SO_4$ was added in amounts so as to give concentrations of 1 mM (28 $\mu g^{-N}/ml$), 2 mM (56 $\mu g^{-N}/ml$), 3 mM (84 $\mu g^{-N}/ml$), 3.8 mM (106 $\mu g^{-N}/ml$), 4 mM (112 $\mu g^{-N}/ml$) and 5 mM (140 $\mu g^{-N}/ml$) in the medium. After sterilization at 15 lb for 20 min the flasks were inoculated with one ml of the 15 days old culture and placed in an incubator at 30°C. The experiment was conducted in triplicate. Nitrite was estimated colorimetrically after every 2 days upto 10 days by procedure described earlier.

MNP was taken at the start and at the end of the experiment by the procedure already described.

Determination of Optimum Substrate Concentration for Nitrobacter sp.:

The effect of different concentrations of nitrite-N on the growth of Nitrobacter sp. was studied in same manner as for Nitrosomonas.

The same basal medium as employed in the previous experiment for Nitrobacter was used.

Thirty ml of the medium was taken in 150 ml Erlenmeyer flasks and $NaNO_2$ was added in amounts so as to give concentrations of 5 mM (69 $\mu g^{-N}/ml$), 7.5 mM (103.4 $\mu g^{-N}/ml$), 10 mM (138 $\mu g^{-N}/ml$), 14.5 mM (200 $\mu g^{-N}/ml$), 15 mM (207 $\mu g^{-N}/ml$),

20 mM (276 µg/ml) in the medium. After sterilization the medium was inoculated with one ml of 15 days old inoculum and incubated at 30°C.

Nitrate formation was estimated at 3 days interval upto 15 days by salicylic acid method as described earlier (Cataldo et al., 1975). MPN was also determined in the start and at the end of the experiment in the same way as in previous experiment.

Determination of the Effect of Fungicides on Nitrosomonas sp. and Nitrobacter sp. in liquid culture :

Effect of different concentrations of fungicides zineb and captafol on the growth and activity of Nitrosomonas and Nitrobacter was studied in pure culture in triplicate.

Thirty ml of the recommended medium was taken in 250 ml of Erlenmeyer flask. After sterilization zineb and captafol were added in the medium in the concentrations ranging from 0 to 10 µg per ml of the medium. One ml of 15 days old inoculum was added to each flask. Flasks were incubated at 30°C in a rotary shaker. Observation of nitrite production and nitrate production were taken at three days interval. Experiment was performed upto 15 days and was conducted in triplicate.

MPN counts of Nitrosomonas sp. and Nitrobacter sp.

were taken in the beginning and at the end of the experiments. The procedure was same as described in previous experiment.

Determination of the Effect of Fungicides on Nitrification in Soil :

Two hundred g of air dried soil was taken in plastic containers in triplicate. Nitrogen was added in the form of $(NH_4)_2SO_4$ at the rate of 200 μg^{-N} per g soil.

Solutions containing different concentrations of zineb and captafol were made in dimethyl sulfoxide (DMSO). The solution was made in such a manner so that the amount of DMSO added with the fungicides was constantly 1 ug per g soil. The fungicides were dissolved in DMSO, so that they could be uniformly mixed in the soil. Water was added to 60% of the water holding capacity and three replicate sets were inoculated at 30°C. Mixing was done at alternate days and water content was adjusted every week. A control without ammonium sulphate was also maintained.

NH_4^+ - N, NO_2^- - N and NO_3^- - N in the soil were estimated every week by steam distillation method (Keeney and Bremner, 1966) and MPN counts for both Nitrosomonas and Nitrobacter were taken. All determinations were carried out in triplicate and values are the average of

three readings.

Determination of the Effect of Fungicides on Mineralization of Urea-N in soil :

This experiment was performed in the manner similar to the previous experiment.

Two hundred g of soil was taken in plastic containers. Urea granules were added as nitrogen source at a concentration of 200 $\mu g \frac{N}{g}$ soil.

Urea Coating : Urea granules of 2-3 mm size were coated with fungicides (Zineb and Captafol) with the help of coaltar. Different quantities of fungicides were weighed and added to 840 mg urea granules so as to arrive at required fungicide concentration. To this mixture 0.5 ml of coaltar solution (prepared by dissolving 5 g of coaltar in 50 ml of toluene) was added and mixed well so that fungicides adhered well to urea granules. The toluene was allowed to evaporate and the required amount of coated granules were added to soil and mixed well. Controls without coaltar and urea coated with coaltar only were also kept. The moisture was adjusted to 60% of the water holding capacity and incubation was done at 30°C.

The soil samples were removed at weekly intervals and the NH_4^+-N, NO_2^--N and NO_3^--N were estimated.

MPN for Nitrosomonas and Nitrobacter in the soil was also monitored at 0 day and after two and four weeks.

Determination of NH_4^+ - N, NO_2^--N and NO_3^--N in soil :

Preparation of Soil Extract: Ten g of soil from each container was taken in 250 ml conical flask and 100 ml of 2 M KCl was added. The flasks were kept on a rotary shaker for one hour. The suspension was filtered through Whatman No.42 filter paper and the filtrate was stored in a refrigerator until analysis could be performed.

Steam distillation procedure for determination of NH_4^+ - N, NO_2^- - N and NO_3^- - N with MgO and Devarda's alloy as described by Keeney and Bremner (1966) was employed.

Reagents :

(a) Magnesium oxide (MgO): Heavy MgO in an electric muffle furnace was heated at 600°- 700°C for two hours and then stored in a tightly stoppered bottle.

(b) Boric acid-Indicator solution: Dissolved 20 g boric acid in about 700 ml of hot water and transferred the cooled solution to one litre volumetric flask containing 200 ml of mixed solution (prepared by dissolving 0.300 g of bromocresol green and 0.165 g of methyl red in 500 ml of ethanol). After mixing the contents of the

flask 0.05 N NaOH was added until a colour change from pink to pale green appeared when 1 ml of the solution was mixed with 1 ml of water. The solution was thus diluted to one litre with water and mixed thoroughly.

(c) Devarda's alloy: A 100-mesh powder of the alloy was used.

(d) Sulfamic acid: Dissolved 2 g of sulfamic acid in 100 ml of water and stored the solution in a refrigerator.

(e) Sulfuric acid (H_2SO_4); 0.005 N.

Procedure :

(a) Ammonium-Nitrogen:

Five ml of boric acid indicator solution was added to a 50 ml Erlenmeyer flask that was marked to indicate a volume of 30 ml and the flask was placed under the condensor of the steam distillation apparatus. An aliquot (10 ml) of the soil extract was added into the distillation flask, and 0.2 g of MgO was added. The flask was attached to the steam distillation apparatus. Distillation was started by closing the stopcock on the steam by pass tube of the distillation apparatus. When the distillate reached the 30 ml mark on the receiver flask,

the distillation was stopped by opening the stopcock on the steam by pass tube; the end of the condensor was rinsed and NH_4^+-N in the distillate was determined by titration with 0.005 N H_2SO_4 from a micro-burette (1 ml of 0.005 N H_2SO_4 equals 70 µg of NH_4^+ - N). The colour change at the end point was from green to a permanent faint pink.

(b) (Nitrate + Nitrite) - Nitrogen :

After removal of NH_4^+-N from the sample as described above, the stopper was removed from the side arm of the flask. The flask was allowed to cool and then 2.0 g of Devarda's alloy was added rapidly and the stopper was replaced immediately in the neck of the side arm. The distillation was done in the same manner as described for NH_4^+-N. Then the nitrate and nitrite nitrogen was determined by titrating the distillate with 0.005 N H_2SO_4.

(c) Nitrate-Nitrogen :

The procedure described for determination of (NO_3^- + NO_2^-)-N was followed but the analysis was performed on a sample that has been treated with 1 ml of sulfamic acid to destroy nitrite before the addition of MgO and Devarda's alloy.

MPN count of *Nitrobacter* and *Nitrosomonas* :

For MPN determination, 10 g of soil sample was taken from each container and added in 90 ml of sterilized phosphate buffer. Then serial dilutions were made in phosphate buffer (pH - 7.0) under aseptic conditions.

Media for *Nitrosomonas* and *Nitrobacter* were prepared. Each medium (4 ml) was distributed in tubes and sterilized. One ml from each serial dilution was inoculated to 4 ml of sterilized medium contained in tubes which were incubated at 30°C in dark. Spot test for the presence of both *Nitrosomonas* and *Nitrobacter* were performed after three weeks by the method described earlier and MPN counts were calculated from the MPN tables.

CHAPTER - IV

RESULTS AND DISCUSSION

The importance of the studies on the effect of pesticides on non-target organisms has been realized long back and detailed reports are available on the effect of these on different soil microbiological processes. The common drawback however observed in these studies on the effect of pesticides on soil microbiological processes has been that either the experiments have been done with pure culture or the effect has been studied directly in the soil. Little data is available to indicate the correlation between pure culture and soil studies. Whereas the pure culture may provide ideal conditions for growth of the organisms and the toxicants may have direct effect on the microorganisms, on the other hand in soil the conditions are rarely ideal for growth but the toxicant may be subjected to absorption/adsorption and degradation. During the present investigation, therefore, the following studies were conducted so as to ascertain the effect of zineb and captafol on nitrifying bacteria and nitrification process in soil and to examine the possibility of their use as nitrification inhibitors.

1. Growth studies of Nitrosomonas and Nitrobacter to determine the growth pattern and optimum substrate concentration.

2. Effect of two fungicides (zineb and captafol) on Nitrosomonas and Nitrobacter in liquid culture.

3. Effect of fungicides on nitrification of $(NH_4)_2SO_4$ and mineralization of urea in soil.

Growth Pattern of Nitrifying Bacteria :

The growth pattern of Nitrosomonas and Nitrobacter isolates used during present investigation was studied so as to determine the proper incubation period for further experiments. The substrate oxidation or product formation has been considered as a function of the number of nitrifiers in determining the growth pattern.

Nitrosomonas :

The results given in Fig.1 show the formation of nitrite by Nitrosomonas. It has been observed that the bacterium grew only upto six days, afterwards there was no growth. There was increase in nitrite from first day upto sixth day, but there was no increase later. Only 10-12% of the added NH_4^+-N was oxidized to NO_2^--N in 10 days. Nitrite has been shown to be inhibitory for many

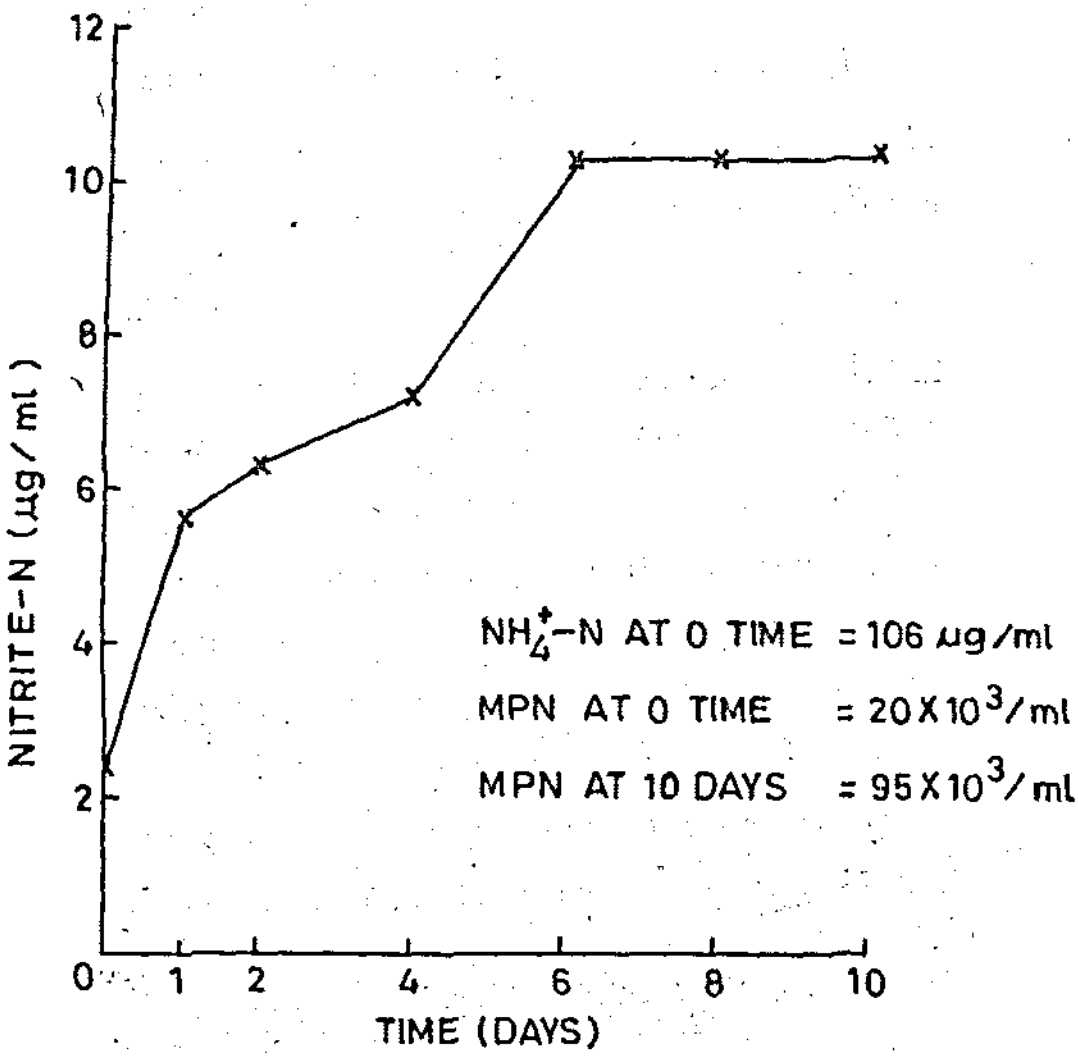


FIG.1 GROWTH PATTERN OF NITROSOMONAS sp

microorganisms (Cassens et al., 1978) and it is likely that it inhibits Nitrosomonas as well. Therefore, there was no oxidation of NH_4^+-N when the nitrite-N concentration reached 10-12 µg/ml in the medium.

The MPN revealed that the number of cells increased from 20 x 10^3/ml to 95 x 10^3/ml after ten days.

Nitrobacter :

As against Nitrosomonas, the Nitrobacter grew upto 15 days (Fig.2) and the conversion of NO_2^--N to NO_3^--N was about 95% during this period. Nitrate is known to accumulate in soils upto several hundred ug/g without any adverse effect. The MPN counts at O time and after 15 days revealed that the population increased about 100 folds in 15 days. The increase was relatively less as compared to other heterotrophic bacteria since these bacteria are well-known to be slow growing (Bock, 1978).

Effect of substrate concentration on activity of nitrifying bacteria :

The effect on ammonium oxidation :

The effect of different concentrations of $(NH_4)_2SO_4$ in the medium was studied to determine the optimum concentration for growth of Nitrosomonas. The $(NH_4)_2SO_4$ concentration as recommended in the medium by Belser and Schmidt

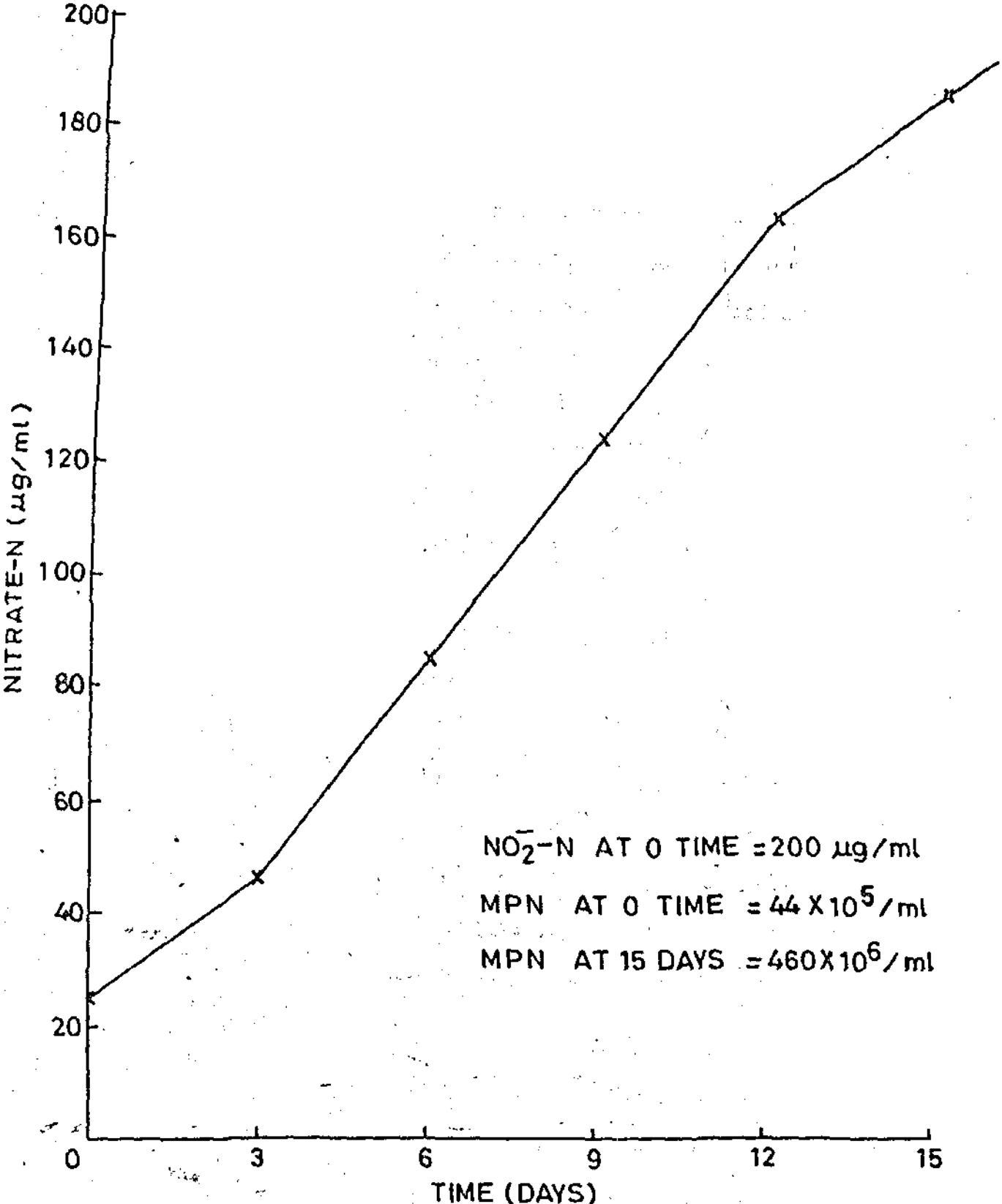


FIG. 2 GROWTH PATTERN OF NITROBACTER sp

(1978) was 3.8 mM (106 µg/ml NH_4^+-N).

It was observed that this isolate of Nitrosomonas also showed maximum growth (as observed by conversion of NH_4^+-N to NO_2^--N) in the medium containing 3.8 mM $(NH_4)_2SO_4$ (106 µg/ml NH_4^+-N) (Table 3). There was not much difference in the nitrite production in the medium containing 3.8 mM or 4 mM-$(NH_4)_2SO_4$. About 15% of NH_4^+-N was converted to NO_2^--N in 4 days and there was no oxidation of NH_4^+-N lateron. The NO_2^--N formation from the medium containing either 28 µg/g NH_4^+-N or 140 µg/g NH_4^+-N was not much different. This confirmed the earlier observation that nitrite is inhibiting above a certain level. Higher concentration of NH_4^+-N present in the form of NH_3 has been reported to be inhibitory for Nitrosomonas at pH 8 and above (Smith, 1964).

The MPN count of Nitrosomonas rather decreased at low concentration of NH_4^+-N but it increased at the optimum substrate concentration, however, there was no significant difference in the population between 3 to 4 mM of $(NH_4)_2SO_4$.

Effect on Nitrite Oxidation :

The $NaNO_2$ concentration as recommended in medium by Belser (1977) is 14.5 mM (200 µg/ml NO_2^--N).

It was observed in this experiment also (Table 4) that growth and activity of Nitrobacter sp. was maximum

Table - 3

Effect of substrate concentration on nitrite production by Nitrosomonas sp.

Concentration of substrate $(NH_4)_2SO_4$	Period of Incubation(Days) 2	4	6	8	10	MPN** on 10th day (cells/ml)
	NO_2^- - N (µg/ml)*					
1 mM (28 µg N/ml)	8.2	11.6	11.6	11.9	11.6	9×10^3
2 mM (56 µg N/ml)	8.6	11.9	11.9	11.6	11.9	9×10^3
3 mM (84 µg N/ml)	9.0	12.8	12.7	12.8	13.0	20×10^4
3.8 mM (106 µg N/ml)	9.4	14.4	14.4	14.6	14.9	47×10^4
4 mM (112 µg N/ml)	9.4	12.7	12.5	13.0	13.7	47×10^4
5 mM (140 µg N/ml)	9.0	11.6	11.9	13.0	13.4	20×10^3

* NO_2^- - N at T_o was 3.41 µg/ml.

** MPN at T_o was 28×10^3/ml.

Table - 4

Effect of substrate concentration on nitrate production by Nitrobacter sp.

Concentration of substrate ($NaNO_2$)	Period of Incubation(Days) 3	6	9	12	15	MPN** on 15th day (cells/ml)
			NO_3^- - N (µg/ml)*			
5 mM (69 µg N^-/ml)	5.0	36.0	45.5	66.0	66.0	28×10^7
7.5 mM (103.4 µg N^-/ml)	10.0	39.0	77.0	92.0	97.0	35×10^7
10 mM (168 µg N^-/ml)	10.5	59.0	108.0	120.0	128.0	42×10^7
14.5 mM (200 µg N^-/ml)	10.5	85.5	122.0	165.0	195.0	42×10^7
15.0 mM (207 µg N^-/ml)	9.0	85.0	119.0	165.0	192.0	35×10^7
20.0 mM (276 µg N^-/ml)	55.0	70.5	75.0	140.0	150.0	14×10^7

* The NO_3^- - N at t_o was 2.5 µg/ml

** The MPN at t_o was 95×10^5/ml.

when the concentration of $NaNO_2$ was 14.5 mM (200 µg/ml NO_2^--N). The nitrate formation increased as the concentration of NO_2^--N increased upto 15 mM of sodium nitrite.

As observed in the earlier experiment on growth pattern of Nitrobacter, more than 95% of NO_2^--N was converted to NO_3^--N in all the treatments except the medium containing 20 mM sodium nitrite (276 µg/ml NO_2^--N).

The MPN of Nitrobacter increased at the optimum substrate concentration. The increase was not upto the same extent at lower or higher substrate concentrations but the number of cells increased at all concentrations of sodium nitrite. The optimum substrate concentrations observed during this experiment were used in further experiments.

Effect of fungicides (zineb and captafol) on nitrification in liquid culture :

The effect of different concentrations of zineb and captafol on the oxidation of NH_4^+-N by Nitrosomonas and on the oxidation of NO_2^--N by Nitrobacter are reported in Tables 5-8. The MPN of both the bacteria were determined at 0 time and at the end of incubation period.

Effect of zineb :

Nitrosomonas : It is observed from Table 5 that zineb

Table - 5

Effect of zineb on Nitrosomonas in liquid culture

Concentration of zineb (µg/ml)	Period of Incubation(Days) 3	6	9	12	MPN*** on 12th day (cells/ml)
	NO_2^- - N (µg/ml)*				
0	8.05**	9.60	9.66	9.66	75×10^3
1	1.29	1.44	1.46	1.50	9×10^3
2.5	1.30	1.34	1.38	1.50	9×10^3
5	1.30	1.34	1.34	1.33	7×10^3
7.5	1.29	1.29	1.32	1.33	19×10^2
10	1.29	1.32	1.29	1.33	16×10^2

* NH_4^+ - N added at t_o - 106 µg/ml

** NO_2^- - N at t_o was 1.29 µg/ml

*** MPN at t_o was 28×10^3/ml

Table - 6

Effect of zineb on *Nitrobacter* in liquid culture

Concentration of zineb (µg/ml)	Period of Incubation(Days) 3	6	9	12	15	MPN*** on 15th day (cells/ml)
			NO_3^- - N (µg/ml)**			
0	7.5*	12.5	34.0	86.5	107.5	11×10^6
1	10.0	12.5	22.5	45.0	100.0	11×10^6
2.5	10.0	10.5	22.4	29.0	45.0	15×10^5
5	9.0	9.0	10.0	27.5	29.0	11×10^4
7.5	10.0	12.5	9.5	21.5	21.0	93×10^1
10	5.5	9.0	9.0	12.5	20.0	11×10^1

* NO_2^- - N added at t_o - 200 µg/ml

** NO_3^- - N at t_o was 5 µg/ml

*** MPN count at t_o was 42×10^4 cells/ml.

Table - 7

Effect of captafol on Nitrosomonas in liquid culture

Concentration of captafol (µg/ml)	Period of Incubation(Days) 3	6	9	12	MPN*** count at 12th day (cells/ml)
	NO_2^- - N (µg/ml)**				
0	8.85*	9.66	10.00	10.40	75×10^3
1.0	8.85	9.60	10.00	10.30	20×10^3
2.5	8.05	9.38	9.60	9.60	28×10^3
5.0	3.89	8.64	8.96	9.30	14×10^3
7.5	2.45	8.21	8.74	9.30	14×10^3
10.0	1.62	8.21	8.64	8.74	14×10^3

* NH_4^+ - N added at t_o - 106 µg/ml

** NO_2^- - N at t_o was 1.29 µg/ml

*** MPN count at t_o was 28×10^3/ml

Table - 8

Effect of captafol on Nitrobacter in liquid culture

Concentration of captafol (µg/ml)	Period of Incubation (Days)					MPN*** count on 15th day (cells/ml)
	3	6	9	12	15	
	NO_3^- - N (µg/ml)**					
0	15.0*	30.0	75.0	120.0	120.5	46×10^7
1.0	12.5	29.0	85.5	105.0	111.0	39×10^7
2.5	10.0	27.5	75.0	95.0	105.0	39×10^6
5.0	10.0	20.5	45.0	76.0	96.0	15×10^4
7.5	7.5	12.5	42.0	72.0	95.0	28×10^3
10.0	5.0	7.5	34.0	60.0	76.0	15×10^2

*NO_2^- - N added at to - 200 µg/ml

**NO_3^- - N at to was - 5.0 µg/ml

***MPN count at to was 42×10^4/ml

is inhibitory to Nitrosomonas even at a concentration of 1 µg/ml in the culture medium. The inhibition of nitrification was observed from the third day upto 12th day. Whereas, in control, having no zineb, about 10% of NH_4^+-N was converted to NO_2^--N but in the medium containing different concentrations of zineb there was almost no formation of nitrite.

MPN count after 12 days was also found to have decreased even at 1 µg/ml concentration of zineb. In control the MPN count was 75×10^3 cells/ml after 12 days but in medium containing 1 µg/ml zineb the MPN was 9×10^3 cells/ml. The MPN further decreased (16×10^2) with increasing concentrations of zineb.

Nitrobacter : The inhibitory effect of zineb on Nitrobacter was less as compared to that on Nitrosomonas (Table 6). At 1 µg/ml concentration of zineb in the medium, the nitrite oxidation was slow upto 12 days but afterwards, the rate of oxidation increased. Zineb at 5 µg/ml concentration and above was very inhibitory for nitrite oxidation. The nitrite oxidation was about 40% as compared to control in the presence of 2.5 µg zineb/ml in the medium.

The MPN increased about 250 folds after 15 days in control and at 1 ug zineb/ml but it decreased significantly

at 7.5 and 10 µg concentrations.

Zineb was thus bactericidal for both Nitrosomonas and Nitrobacter.

Effect of Captafol :

Nitrosomonas : It has been observed that captafol was not as inhibitory to Nitrosomonas as zineb (Table 7). Significant inhibition by captafol was observed on third day at 5 µg and higher concentrations in the medium, but the oxidation increased after 3rd day even at these concentrations. The nitrite formation after 12 days in presence of 10 µg captafol was about 80% of that observed in control.

The MPN of Nitrosomonas increased three-folds in control, but there was no increase in presence of captafol. The bacterium did not multiply in presence of captafol although it remained active.

Nitrobacter : Captafol was found to inhibit Nitrobacter activity at a concentration of 5 µg/ml and above (Table 8). About 60% of NO_2^--N was oxidized to NO_3^--N in 15 days in control, but the oxidation in presence of 5 µg/ml captafol in the medium was about 48% and in presence of 10 µg/ml captafol, it was only about 38%. The rate of oxidation at higher concentrations of captafol was slow for the

first 6 days, but it increased later which was probably due to the adaptation by the bacterium. Many microorganisms are known to adopt to the presence of toxicants in the environment (Hill and Wright, 1978).

The MPN of Nitrobacter increased in control and in presence of 1 and 2.5 µg/ml of captafol but it drastically decreased in presence of 7.5 and 10 µg of captafol. This fungicide was thus bactericidal for Nitrobacter at higher concentrations whereas at lower concentrations, it had no adverse effect.

Effect of fungicides on nitrification of $(NH_4)_2SO_4$ in soil:

Pesticides on addition to soil are subjected to absorption/adsorption and degradation to different degree depending upon the agroclimatic conditions. Their effect on soil microorganisms may be different in soil to that observed under pure culture conditions (Alexander, 1978; Anderson, 1978). Nitrification has been observed to be the most sensitive soil microbiological process to many of the agricultural chemicals commonly used (Atlas et al.,1978). The effect of zineb and captafol on their direct addition to the soil on nitrification of nitrogen from $(NH_4)_2SO_4$ and on MPN of Nitrosomonas and Nitrobacter has been studied

and the results are given in Tables 9 to 14. This soil contained organic C 0.42-0.48% total N 0.065-0.072% and had a pH of 7.6-7.8.

Effect of zineb:

The results given in Table 9 show that zineb on addition to soil had significantly adverse effect on oxidation of NH_4^+-N only at 10 and 20 ug/g. Dimethyl sulfoxide (DMSO) which was used as a solvent for the fungicides, itself decreased nitrification upto 2 weeks, but after 4 weeks, the amount of nitrate formed was similar in presence of $(NH_4)_2SO_4$ alone or along with DMSO. The dimethyl sulphoxide has earlier been reported to the toxic for many eucaryotic and procaryotic algae (Voight and Lynch, 1974). The nitrate formed in presence of 10 and 20 ug/g of zineb was respectively 70 and 66% to that of control with $(NH_4)_2SO_4$. The inhibition for the first two weeks was more in presence of 10 and 20 ug/ml of zineb, but later the nitrifying bacteria get adapted to the conditions and nitrification process continued. The decline in nitrate formation in presence of 2.5 and 5.0 ug zineb could be attributed only to the presence of DMSO.

The MPN of Nitrosomonas (Table 10) increased significantly in presence of $(NH_4)_2SO_4$ for two weeks and then decreased. There was significant decline in population in presence of DMSO for two weeks, but it increased later. The

Table - 9

Effect of zineb on nitrification in soil

S.No.	Treatment	Period of Incubation (Weeks)							
		1		2		3		4	
		N (µg/g soil)							
1.	Soil only*	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N
		28.0	112.0	28.0	133.0	21.0	119.0	21.0	119.0
2.	1 + $(NH_4)_2SO_4$	114.0	175.0	85.0	210.0	91.0	238.0	84.0	231.0
3.	2 + DMSO	189.0	126.0	168.0	154.0	119.0	210.0	84.0	238.0
4.	3 + Zineb 2.5 µg/g	189.0	126.0	168.0	154.0	126.0	189.0	91.0	231.0
5.	3 + Zineb 5 µg/g	196.0	126.0	175.0	154.0	133.0	189.0	91.0	217.0
6.	3 + Zineb 10 µg/g	210.0	112.0	196.0	126.0	182.0	141.0	147.0	168.0
7.	3 + Zineb 20 µg/g	217.0	112.0	210.0	119.0	203.0	126.0	163.0	159.0

* The NH_4^+-N and NO_3^--N were 63 µg/g and 98 µg/g in soil respectively when observed at To. These were 238 µg/g and 98 µg/g respectively when $(NH_4)_2SO_4$ was added in soil.

Table - 10

Effect of zineb on MPN of *Nitrosomonas* during nitrification

S.No.	Treatment	MPN during Incubation period(Weeks) 1	2	4
		(Cells/g soil)		
1.	Soil only*	150×10^3	150×10^3	29×10^3
2.	1 +$(NH_4)_2SO_4$	210×10^3	1100×10^3	150×10^3
3.	2 + DMSO	15×10^3	21×10^3	93×10^3
4.	3 + Zineb 2.5 µg/g	15×10^3	15×10^3	150×10^3
5.	3 + Zineb 5 µg/g	15×10^3	9×10^3	43×10^3
6.	3 + Zineb 10 µg/g	7×10^3	15×10^3	43×10^3
7.	3 + Zineb 20 µg/g	7×10^3	15×10^3	43×10^3

* The MPN count at To was 150×10^3/g soil

Table - 11

Effect of Zineb on MPN of *Nitrobacter* during nitrification

S.No.	Treatment	MPN during Incubation period(Weeks)		
		1	2	4
		(Cells/g soil)		
1.	Soil only*	460×10^3	150×10^3	44×10^3
2.	1 +$(NH_4)_2SO_4$	1100×10^3	1100×10^3	1100×10^3
3.	2 + DMSO	43×10^3	21×10^3	21×10^3
4.	3 + Zineb 2.5 µg/g	43×10^3	28×10^3	21×10^3
5.	3 + Zineb 5 µg/g	21×10^3	28×10^3	28×10^3
6.	3 + Zineb 10 µg/g	23×10^3	15×10^3	20×10^3
7.	3 + Zineb 20 µg/g	20×10^3	20×10^3	23×10^3

* The MPN count at To was 210×10^3/g soil

Table - 12

Effect of captafol on nitrification in soil

S.No.	Treatment	Period of Incubation (Weeks)							
		1		2		3		4	
		N (µg/g soil)							
1.	Soil only*	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N
		28.0	112.0	28.0	112.0	21.0	119.0	21.0	119.0
2.	1 + $(NH_4)_2SO_4$	119.0	175.0	105.0	210.0	91.0	238.0	84.0	231.0
3.	2 + DMSO	189.0	126.0	168.0	154.0	119.0	210.0	84.0	238.0
4.	3 + Zineb 2.5 µg/g	189.0	133.0	168.0	161.0	119.0	203.0	77.0	245.0
5.	3 + Zineb 5 µg/g	203.0	119.0	168.0	154.0	85.0	210.0	70.0	259.0
6.	3 + Zineb 10 µg/g	231.0	84.0	217-0	112.0	175.0	140.0	119.0	203.0
7.	3 + Zineb 20 µg/g	217.0	91.0	217.0	98.0	189.0	133.0	133.0	189.0

*The NH_4^+ - N and NO_3^--N were 63 µg/g and 98 µg/g in soil respectively at to and were 238 µg/g and 98 µg/g respectively when $(NH_4^+)_2SO_4$ was added in soil.

Table - 13

Effect of captafol on MPN of *Nitrosomonas* during nitrification

S.No.	Treatment	MPN during Incubation period (weeks)		
		1	2	4
		(Cells/g soil)		
1.	Soil only*	460×10^3	150×10^3	29×10^3
2.	1 +$(NH_4)_2SO_4$	1100×10^3	1100×10^3	150×10^3
3.	2 + DMSO	43×10^3	21×10^3	93×10^3
4.	3 +Captafol 2.5 µg/g	43×10^3	9×10^3	93×10^3
5.	3 +Captafol 5 µg/g	21×10^3	21×10^3	150×10^3
6.	3 +Captafol 10 µg/g	23×10^3	11×10^3	43×10^3
7.	3 +Captafol 20 µg/g	20×10^3	9×10^3	9×10^3

* The MPN count at t_o was 150×10^3/g soil

Table - 14

Effect of captafol on MPN of *Nitrobacter* during nitrification

S.No.	Treatment	MPN during Incubation period (weeks)		
		1	2	4
		(Cells/g soil)		
1.	Soil only*	460×10^3	150×10^3	44×10^3
2.	1+$(NH_4)_2SO_4$	1100×10^3	1100×10^3	1100×10^3
3.	2+DMSO	43×10^3	21×10^3	21×10^3
4.	3 + Captafol 2.5 µg/g	43×10^3	43×10^3	75×10^3
5.	3 + Captafol 5 µg/g	43×10^3	21×10^3	43×10^3
6.	3 + Captafol 10 µg/g	43×10^3	15×10^3	43×10^3
7.	3 + Captafol 20 µg/g	15×10^3	15×10^3	20×10^3

* The MPN count at t_0 was 210×10^3/g soil.

population in presence of 10 and 20 µg/g of zineb also declined in the first two weeks, but increased later. Zineb appeared to have resulted in the killing of some cells initially, but the remaining cells were able to slowly multiply. Slow recovery of bacteria adversely affected on addition of fungicides, has been reported by Audus (1970).

As regards the effect of different concentrations of zineb on Nitrobacter (Table 11) it had no adverse effect. DMSO had a significant adverse effect on Nitrobacter also, but addition of zineb with DMSO did not worsen the negative effect. The Nitrobacter population in control (with $(NH_4)_2SO_4$) increased about four folds, but it decreased manyfolds in presence of DMSO which was definitely bactericidal.

Effect of captafol :

Captafol decreased nitrification to some extent only at 20 µg/g concentration (Table 12). The decline in nitrate formation was observed at 10 µg/g of captafol only upto three weeks. The nitrate formation was similar in presence of DMSO alone or DMSO with 2.5 and 5 µg/g captafol in soil. Therefore, the decline in nitrate formation in the first two weeks could be attributed to the presence

of DMSO. The Nitrosomonas population (Table 13) in soil increased on addition of $(NH_4)_2SO_4$ but it decreased significantly in the first two weeks on addition of DMSO. There was however increase in the fourth week. The addition of 10 and 20 µg/g of captafol with DMSO decreased Nitrosomonas population further.

The Nitrobacter population (Table 14) was not significantly affected at different concentrations of captafol. The population in presence of DMSO alone and in presence of different concentration of fungicide was similar. The decrease in the population was solely due to DMSO since the population had increased manyfolds in the treatment containing only $(NH_4)_2SO_4$.

During the study of the effect of two fungicides on nitrification in soil, no accumulation of nitrite was observed at any stage in any treatment. The absence of nitrite in the treatment containing only ammonium sulfate clearly showed that nitrite was oxidised as soon as it was formed. Russel (1973) reported that under ideal conditions of nitrification, the oxidation of nitrite is more rapid as compared to oxidation of ammonium and hence nitrite accumulation is normally not observed. There was no accumulation of nitrite even on addition of fungicides

showing clearly that the decreased nitrification was not due to the decline in the number of Nitrobacter. The decline in nitrification at higher concentrations of zineb or captafol was thus mainly due to the reduction in the number of Nitrosomonas.

This type of effect of these two fungicides could be a favourable attribute in their choice as nitrification inhibitors because accumulation of nitrite results into phytotoxicity and inhibition of many soil microorganisms (Hauck, 1972). N-serve, the well known nitrification inhibitor inhibits Nitrosomonas at 0.2 µg/ml but it has no adverse effect on Nitrobacter even at 50 µg/ml.

The comparison of the effect of fungicides on nitrification in soil and in pure culture clearly reveals that toxicity was more in pure culture. Less toxicity in soil could be attributed to the absorption/adsorption of fungicides by different components of the soil or to the degradation of these fungicides by other microorganisms in the soil. Anderson (1978) has concluded on the basis of the review of the work done that the effect of pesticides are far less severe in soil than in pure culture. The extent of effect in soil depends on the physico-chemical properties of the soil and climatic factors

(Burns and Audus, 1970; Knight and Denny, 1970).

Effect of Fungicides on Mineralization of Urea-N in soil:

Coatings of various substances are applied to soluble fertilizers either to control the rate of dissolution or to check their transformation so as to control the release of nutrients from the fertilizers. The slow release could be either because the coat substance acts as a barrier and is slowly degraded or acts as an antimicrobial agent (Hauck, 1972). Sulphur coated urea developed by Tennessee valley Authority, USA contains a sealant which prevents rapid transfer of water and a microbicide-pentachlorophenol (Allen et al., 1968). Since the previous experiments had shown that zineb and captafol decreased nitrification at 10 µg/g concentration, their effect on coating over urea granules was examined on formation of nitrate from urea. Coaltar was used as an adhesive with fungicides.

Effect of zineb :

The formation of nitrate from urea was less in presence of coaltar alone and with zineb for the first two weeks (Table 15). It was, however, observed that in treatments containing coaltar, an accumulation of nitrite took place whereas no nitrite accumulated in treatments

Table - 15

Effect of zineb on mineralization of urea-N

S.No.	Treatment	Period of Incubation (Weeks)											
		1			2			3			4		
		N-(µg/g soil)											
		NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N
1.	Soil only*	63.0	0.0	70.0	49.0	0.0	84.0	49.0	0.0	98.0	35.0	0.0	119.0
2.	1 + Urea	140.0	0.0	189.0	91.0	0.0	224.0	77.0	0.0	266.0	77.0	0.0	273.0
3.	2 + Coltar	105.0	70.0	140.0	91.0	35.0	196.0	70.0	0.0	266.0	70.0	0.0	280.0
4.	3 + Zineb 1 µg/g	98.0	63.0	154.0	91.0	28.0	196.0	91.0	0.0	259.0	56.0	0.0	294.0
5.	3 + Zineb 2.5 µg/g	105.0	70.0	140.0	91.0	35.0	203.0	98.0	0.0	224.0	56.0	0.0	294.0
6.	3 + Zineb 5 µg/g	105.0	56.0	154.0	98.0	28.0	189.0	112.0	0.0	245.0	70.0	0.0	280.0
7.	3 + Zineb 7.5 µg/g	119.0	77.0	133.0	112.0	28.0	175.0	105.0	0.0	238.0	91.0	0.0	259.0
8.	3 + Zineb 10 µg/g	133.0	84.0	119.0	133.0	28.0	154.0	98.0	0.0	224.0	91.0	0.0	266.0
9.	3 + Zineb 20 µg/g	182.0	70.0	63.0	161.0	28.0	126.0	147.0	0.0	189.0	91.0	0.0	224.0

* The NH_4^+-N, NO_2^--N and NO_3^--N contents in the soil at to were 77, 0 and 56 µg/g soil respectively.

without coaltar. The amount of nitrite was about the same at all concentrations of zineb and coaltar. Thus, nitrite accumulation could only be attributed to the addition of coaltar. Zineb had no adverse effect on nitrite formation at 2.5 and 5.0 µg/g concentration but resulted in decreased nitrate formation for 2, 3 and 4 weeks at 7.5, 10 and 20 µg/g concentration respectively.

The MPN of Nitrosomonas (Table 16) revealed that this bacterium decreased significantly at 10 and 20 µg/g concentration of zineb for two weeks, but increased later. Coaltar did not have adverse effect on Nitrosomonas.

The accumulation of nitrite during first two weeks revealed inhibition of Nitrobacter by coaltar, but its population increased after two weeks in all the treatments (Table 17). Zineb was thus inhibitory to Nitrosomonas at higher concentration and coaltar was inhibitory to Nitrobacter.

Effect of Captafol :

The effect of captafol on formation of nitrate from urea-N (Table 18) was similar to the effect observed on coating with zineb and coaltar. There was accumulation of nitrite for the first two weeks in treatments containing

Table - 16

Effect of zineb on MPN of Nitrosomonas during mineralization of urea-N

S.No.	Treatment	MPN during Incubation period (weeks)	
		2	4
		Cells/g soil	
1.	Soil only*	100×10^3	93×10^3
2.	1 + Urea	460×10^3	460×10^3
3.	2 + Coaltar	460×10^3	460×10^3
4.	3 + Zineb 1 µg/g	460×10^3	460×10^3
5.	3 + Zineb 2.5 µg/g	460×10^3	290×10^3
6.	3 + Zineb 5 µg/g	290×10^3	460×10^3
7.	3 + Zineb 7.5 µg/g	150×10^3	460×10^3
8.	3 + Zineb 10 µg/g	75×10^3	290×10^3
9.	3 + Zineb 20 µg/g	20×10^3	150×10^3

* The MPN count at To was 150×10^3/g soil

Table - 17

Effect of zineb on MPN of Nitrobactor during mineralization of urea-N

S.No.	Treatment	MPN during Incubation period (weeks)	
		2	4
1.	Soil only*	93×10^3	43×10^3
2.	1 + Urea	1100×10^3	1100×10^3
3.	2 + Coaltar	75×10^3	460×10^3
4.	3 + Zineb 1 µg/g	93×10^3	460×10^3
5.	3 + Zineb 2.5 µg/g	93×10^3	93×10^3
6.	3 + Zineb 5 µg/g	93×10^3	120×10^3
7.	3 + Zineb 7.5 µg/g	43×10^3	93×10^3
8.	3 + Zineb 10 µg/g	21×10^3	75×10^3
9.	3 + Zineb 20 µg/g	43×10^3	93×10^3

* The MPN count at To was 93×10^3/g soil

Table - 18

Effect of captafol on mineralization of urea-N

S.No.	Treatment	Period of Incubation (Weeks)											
		1			2			3			4		
		N - (µg/g soil)											
		NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N
1.	Soil only*	63.0	0.0	70.0	49.0	0.0	84.0	49.0	0.0	98.0	35.0	0.0	119.0
2.	1 + Urea	140.0	0.0	189.0	91.0	0.0	224.0	77.0	0.0	266.0	77.0	0.0	273.0
3.	2 + Coaltar	105.0	70.0	140.0	91.0	35.0	196.0	70.0	0.0	266.0	70.0	0.0	280.0
4.	3 + Captafol 1 µg/g	105.0	63.0	140.0	91.0	42.0	196.0	70.0	0.0	273.0	70.0	0.0	280.0
5.	3 + Captafol 2.5 µg/g	98.0	63.0	140.0	84.0	35.0	189.0	63.0	0.0	273.0	63.0	0.0	280.0
6.	3 + Captafol 5 µg/g	98.0	56.0	133.0	91.0	35.0	182.0	70.0	0.0	266.0	56.0	0.0	294.0
7.	3 + Captafol 7.5 µg/g	91.0	70.0	120.0	77.0	28.0	167.0	77.0	0.0	247.0	70.0	0.0	280.0
8.	3 + Captafol 10 µg/g	98.0	70.0	108.0	91.0	42.0	142.0	77.0	0.0	208.0	99.0	0.0	251.0
9.	3 + Captafol 20 µg/g	91.0	70.0	98.0	77.0	28.0	129.0	70.0	0.0	190.0	125.0	0.0	225.0

* The NH_4^+-N, NO_2^--N and NO_3^--N contents in the soil at to were 77, 0 and 56 µg/g soil respectively.

coaltar and the presence of captafol with coaltar had no additional effect. Captafol decreased nitrate formation significantly upto 4 weeks only at 20 µg/g concentration. About 67% of the nitrate nitrogen present in control was found in treatment containing 20 µg captafol/g soil. Even 10 µg captafol decreased nitrate formation upto three weeks and nitrate formed was about 60% of the control with urea.

The MPN of Nitrosomonas increased on addition of urea but captafol upto 5 µg/g had no adverse effect but at high concentrations the bacterial count decreased (Table 19). As observed earlier the Nitrobacter count decreased due to presence of coaltar and also at higher concentration of captafol (Table 20) but no accumulation of nitrite was detected beyond three weeks in any treatment.

The results with both zineb and captafol thus showed that these are effective for 3 weeks at 10 µg/g. It may therefore be worthwhile to test their efficiency on plants. The coaltar extract has earlier been used as a coating substance and found to inhibit nitrification for two weeks (Reddy and Prasad, 1975). It was also observed during the studies that more than 90% of urea-N was converted to NH_4^+-N and NO_3^--N in one week. This is in confirmity with the earlier observation (Fisher and Parks, 1958; Bundy and Bremner, 1974; Sahrawat, 1980).

Table - 19

Effect of captafol of Nitrosomonas during mineralization of urea-N

S.No.	Treatment	MPN during Incubation period (week)	
		2	4
1.	Soil only*	100×10^3	93×10^3
2.	1 + Urea	460×10^3	460×10^3
3.	2 + Coaltar	460×10^3	460×10^3
4.	3 + Captafol 1 µg/g	460×10^3	460×10^3
5.	3 + Captafol 2.5 µg/g	290×10^3	460×10^3
6.	3 + Captafol 5 µg/g	290×10^3	460×10^3
7.	3 + Captafol 7.5 µg/g	150×10^3	150×10^3
8.	3 + Captafol 10 µg/g	150×10^3	28×10^3
9.	3 + Captafol 20 µg/g	75×10^3	28×10^3

* The MPN count at t_o was 150×10^3/g soil

Table - 20

Effect of captafol on MPN of Nitrobacter during mineralization of urea-N

S.No.	Treatment	MPN during Incubation period (Weeks)	
		2	4
1.	Soil only*	93×10^3	43×10^3
2.	1 + Urea	1100×10^3	1100×10^3
3.	2 + Coaltar	75×10^3	460×10^3
4.	3 + Captafol 1 µg/g	93×10^3	460×10^3
5.	3 + Captafol 2.5 µg/g	93×10^3	460×10^3
6.	3 + Captafol 5 µg/g	28×10^3	460×10^3
7.	3 + Captafol 7.5 µg/g	43×10^3	460×10^3
8.	3 + Captafol 10 µg/g	43×10^3	75×10^3
9.	3 + Captafol 20 µg/g	43×10^3	43×10^3

* The MPN count at t_0 was 93×10^3/g soil

So far little information is available on the mechanism of the effect of various chemicals on nitrifying bacteria. The inhibition could be caused by interfering with respiration and cytochrome oxidase function, by chelating essential metal ions, or by liberation of toxic compounds. N-serve chelates Cu^{+2} needed for the enzyme responsible for oxidation of ammonium to hydroxylzmine (Stevenson et al., 1982). Therefore, there is a need for a detailed study of the mechanism. Any chemical to be a successful nitrification inhibitor should inhibit nitrification in soil at concentrations below 10 µg/g, should inhibit only Nitrosomonas and should not be toxic to other microorganisms and plants.

CHAPTER - V

SUMMARY

The effect of two fungicides namely zineb and captafol on nitrifying bacteria (Nitrosomonas sp. and Nitrobacter sp.) in liquid culture and on nitrification process in soil was studied to assess the possibility of their use as nitrification inhibitors and the following results were obtained:

1- Zineb inhibited Nitrosomonas even at a concentration of 1 µg/ml in the culture medium but inhibited Nitrobacter only at 5 µg/ml and above. Captafol inhibited Nitrosomonas and Nitrobacter at higher concentrations (7.5 and 10 µg/ml).

2- Zineb inhibited nitrification process significantly in soil at 10 and 20 µg/g concentration. The decrease in nitrification by captafol was less as compared to zineb. The Nitrosomonas population declined during the initial phase of inhibition. Dimethyl sulfoxide which was used as a solvent for these fungicides also reduced nitrification at 1 µg/g but the decline lasted only for two weeks at this concentration.

3- The coating of urea granules with zineb and captafol using coaltar as an adhesive resulted in decreased formation of nitrate from urea. Coaltar decreased the population of

Nitrobacter resulting in accumulation of nitrite.

The concentrations of 10 to 20 µg/g zineb or captafol were thus found effective in decreasing nitrification and could be tried further in plant experiments.

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* Original not seen.