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THESIS

INVESTIGATION ON THE NITRIFICATION INHIBITION POTENTIAL OF SOME ETHIOPIAN MEDICINAL HERBS FOR IMPROVING N-USE EFFICIENCY AND YIELD OF WHEAT

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A laboratory investigation was made to determine the nitrification potential of some soils occurring in the southern and central Ethiopia. Soil samples were collected from eight sites which are diverse in terms of climate and soil type. One hundred gram of processed soil from each sample was transferred into 250 ml capacity plastic cups and amended with 50 $mgkg^{-1}$ of ammonium sulphate and incubated for 2, 3 and 6 weeks. The experiment was laid out in completely randomized design with 3 replications and was conducted in the National Soil Research Laboratory, Addis Ababa, Ethiopia. At the end of each incubation period samples were taken and analyzed for pH, ammonium-N and nitrate-N. The result revealed that the soils varied significantly ($P<0.001$) in their nitrifying ability. The highest amount of nitrate-N was produced by Ziway clay loam (Fluvi-vitric Andisol), Alaba clay loam (Plani-vertic Cambisol), Yirgalem clay loam (Eutric Nitisol), Awassa clay loam (Eutric Fluvisol) and Debreziet loam (Vitric Andisol) soils at the end of 4 weeks of incubation. These soils are comparatively classified as fast nitrifying soils suggesting that there is a high possibility of N loss from these soils. On the contrary, Kokat loam (Dystric Nitisol), Areka clay loam (Haplic Alisol) and Dilla silty (Rhodic ferallsol) soils produced maximum amount of nitrate-N at the end of 6 weeks of incubation and are categorized as slow nitrifying soils. Amending soils with ammonium sulphate stimulated nitrification in all soils. There was a fall in pH of all soils as nitrification proceeds and it was more drastic for amended soils than unameded ones. The variation in nitrifying ability of these soils could be attributed to their differences in soil initial pH, %BS, K, Na and C/N ratio.

Some Ethiopian traditional medicinal herbs along with neem and commercial inhibitors were screened to study their nitrification inhibition ability. Alcohol extract of each herb was applied at 1% rate of dry soil from Awass area and incubated for various periods. N-serve [(2-Chloro (Trichloromethyl) Pyridine)] and Dicyandiamide (DCD) were applied at 20 and 100 μg^{-1} of soil, respectively. The result showed that 72% of tested herbs conserved significantly ($P<0.001$) high amount of NH_4^+ at the end of 2nd week. But among Ethiopian medicinal herbs only *Artemisia afra*, *Echinops spp* and *Eugenia caryophyllata* inhibited nitrification at the end of 3rd week. On the average these herbs inhibited nitrification by 33, 37 and 64%, respectively. *Eugenia caryophyllata* performed as effective as neem but none of them out performed commercial inhibitors. Both effective herbs and commercial inhibitors prevented the soil from acidification that is resulting from nitrification of ammonim-N in the soil

Experiment was conducted to study the interaction between two soil types and inhibitors on nitrifications. It was found that the highest percent nitrification inhibition (45%) was achieved in Ziway soil than Awass (37%) at the end of 2 weeks of incubation indicating that the use of nitrification inhibitors is more beneficial in Ziway than Awassa soil. Inhibitor by soil type interactions were significant indicating that there is a need to match a specific inhibitor for specific soil.

A greenhouse experiment was conducted to investigate the effect of nitrification inhibitors on the grain and straw and yield and yield components and N-recovery of wheat. A 5 X 5 factorial experiment consisting of 5 kinds of inhibitors and 5 levels of nitrogen fertilizer in the form of ammonium sulphate were laid out in RCB design. The inhibitors used were *E.caryophyllata*, neem, N-serve and DCD. It was found that the inhibitors increased the grain yield by 7, 7, 10 and 13% over the control and straw yield by 14.8, 12, 16 and 28.6% over the control respectively. Similarly they increased the N recovery from 60% in the control to 78, 79, 82 and 90% respectively. Application of increasing levels of N fertilizer increased all parameters taken significantly. Inhibitors by N levels interaction were not significant. This implies that the effects of inhibitors are not affected by the change in the levels of N and vice-versa.

Student's signature

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Thesis Advisor's signature

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Wassie Haile Woldeyohannes

February, 2006

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INTRODUCTION

Ethiopia is located in east Africa, between 3^0 and 15^0 N and 33^0 and 48^0 E. It has a total land area of 122.2 million hectares, 65% of which is suitable for crop production. However, only 15% of the total land area is under cultivation currently. The country has 3.5 million hectares of potentially irrigable land out of which only 160,000 hectares are developed to date. Agriculture remains to be the corner stone of Ethiopian economy in which 85% of the population depend in the sector for its lively hood. It accounts for 50% of GDP and 45% of foreign exchange earnings. Despite, the huge working force involved in agriculture and the enormous potential the country has it is unable to produce enough to feed its ever-increasing population. The reasons are diverse and complex. Inadequate use of modern agricultural technologies, high population pressure, recurrent draught and declining soil fertility are some of the factors that account for low crop production and productivity (AHI, 2001).

Declining of soil fertility resulting from erosion, continuous cultivation, deforestation, inadequate incorporation of manure and removal of crop residues to be used as fuel coupled with low inherent soil fertility are a major challenge facing Ethiopia, threatening the food security and self-sufficiency of the country (EARO, 2001; Paulos, 2001). Deficiency of nitrogen is one of the most prominent soil fertility problems contributing significantly to the low crop production. Nitrogen has been mined for many years without adequate replenishment. Traditional methods of nitrogen restoration such as fallowing and crop rotation are practiced little these days as the size of arable land per capita is shrinking with time owing to high population pressure. The situation is very severe in the Ethiopian highland where the population density is quit high.

In an effort to overcome the nitrogen deficiency and increase crop yield, application of inorganic fertilizers has been initiated four decades ago. They were first introduced in 1967 and their consumption increased from 14000 mt in 1974/75 to 50,000 mt in 1997/98. The annual consumption surpassed 200,000 mt in 1993/94 (UNDP, 1995). This figure has grown to 446, 000 mt in 2000 (Taye *et al*., 2002). In the majority of Ethiopian soils the responses of crops to the application of fertilizers have been significant and enormous increase in the yield of several crops have been obtained. Experimental results of fertilizer trails conducted across the country revealed that the yield of cereals could be increased by more than 100% (Kelsa *et al*., 2001). Despite the increasing cost, currently crop production depends mostly on the application of inorganic fertilizers such as urea and DAP.

Even if application of chemical fertilizers are taken as a major route to boost up crop yield and continued reliance of farmers and state farms in Ethiopia and elsewhere in the world, the N-use efficiency of crops is very low. For instance, in the USA, not more than 50% of the applied nitrogen is utilized by corn in the season (Nelson and Hubner, 2001). In the tropics, only 25-40% of the applied nitrogen is utilized by crops (Sahrawat *et al*., 1977). Research results in Ethiopia revealed that the N-use efficiency of wheat was 31.9 and 42.3% on vertisols and Nitisols respectively (Amsal and Tanner, 2001). Reduced N-use efficiency is due mainly to loss of nitrogen through leaching and denitrification following excess rate of nitrification.

Nitrification is a biological conversion of ammonia first to nitrite then to nitrate. This stepwise dissimilation of ammonia is brought about by ammonia oxidizing and nitrite oxidizing bacteria respectively (Coyne, 1999). From agricultural point of view, nitrification is very important in that its end product, nitrate (NO_3^-), is one of the forms of nitrogen taken up by plants (Tsidale *et al*., 1985). However, if it proceeds at higher rate, nitrate will be produced in excess of plant demand. In such case, the nitrate produced will be lost through leaching and denitrification. The net effect of which is reduced N-use efficiency and crop yield. This will ultimately leads to economic loss. Further more, excess nitrate in the soil promotes cation losses and ultimately causes acidification of soils (Potash institute, 1979).

Soils vary in their nitrification potential (Meisinger *et al*., 1980; Schmidt and Bleser, 1994; Degrange *et al*., 1998), depending on factors such as population size of nitrifying bacteria, pH, soil moisture, temperature, mineral nutrients, texture, C/N ratio, plant community, and agricultural management practices (Alexander, 1977; Armsrong and Burt, 1993; Brady and Well, 2002). The occurrence of these factors in optimum condition favors excess nitrification. On the other hand sub optimal condition of any one or more of these factors reduce nitrification. In any case, a soil in which the rate of nitrate production is synchronized with crop demand is important, as it will help to use the N-coming from either organic sources or chemical fertilizers or both effectively.

Studies on the nitrification potential of different soils and associated factors are indispensable. This is because the result of such study will help to categorize soils having high, medium, and low rate of nitrification rate and identify major factors responsible for variation in nitrification. This will ultimately help to design N-management techniques that will minimize its loss. Cognizant of this fact soils of USA are mapped with respect to nitrification potential and soils of western USA have low rate of nitrification rate and hence the N loss through leaching and denitrification is very low. Where as the soils of eastern part is rated as having high rate of nitrification potential (Hoeft, 1984). However, only limited information is available on the nitrification potential of Ethiopian soils so far. Therefore, in the light of continued reliance of the country on inorganic nitrogen fertilizers and the need to use them effectively, it is important to investigate the nitrification potential of some Ethiopian soils.

One of the most popular strategy of reducing excess nitrification and there by increase the N-use efficiency of crops is by the use of nitrification inhibitors (NI) (Hoeft, 1984). Nitrification inhibitors are chemical compounds that block the conversion of ammonium to nitrite by killing or interfering with the metabolic activity of ammonium oxidizing bacteria (Mesinger *et al*., 1980; Hauck, 1984; Nelson and Huber, 2001). They were first introduced in to commercial market in 1976. N-serve

(2-chloro (trichloromethyl) pyridine) was the first NI discovered and commercialized and subsequently more than a dozen of them were developed (Prasad and Power, 1995).

There are several reports indicating that NI increases the N-recovery and yield of crops (Mesinger *et al*., 1980; Hauck, 1984; Prasad and Power, 1995). For instance, Malhi and Nyborg (1982) reported that application of aqua ammonia or urea plus NI increased the yield of barley from 800 kg ha-1 to1370 kg ha^{-1}. Similarly, a 30% increase in grain yield of maize has been found in an experiment conducted in western Corn Belt of USA (Nelson and Huber, 2001). A grain yield increase of maize by over 88% was found due to combined application of anhydrous ammonia and nitrapyrin (Nelson and Hubner, 1980).

There are nitrification inhibitors of plant origin. Many plant products and by products such as neem (*Azadirachta indica*), pyrathrum flowers, karanaja were reported to have nitrification inhibition properties (Sahrawat, 1989). Plant herbs that are rich in phenolic compounds are effective nitrification inhibitors (Rice, 1974). According to Rice and Pancholy (1973), condensed tannin, ellagic acid, gallic acid and digallic acid isolated from oaks and pine were found to inhibit nitrification completely even at very low concentrations. Sahrawat *et al*.,(1977) found that karajin, the major crystalline principle of karaja (*Pongamia glabra* vet) seeds to inhibit nitrification in soil up to 75 days.

Appreciable increase in the grain and dry matter yield due to the application of karanjin has been obtained due to increased N-use efficiency (Sahrawat and Mukerjee, 1977). Ethiopian medicinal herbs appear to have a potential as nitrification inhibitors. Some of them have been studied against bacteria that cause human diseases. Research results indicate that *Vernonia amygadalina*, *Carum copticum* and *Artemisia afra* were effective against *Bacillus cerus* and *Staphylococus aureus* (Mintesinot and Mogessie, 1999).

Thus, it is hypothesized that if these medicinal herbs and others are tested,

they could be effective against ammonium oxidizing bacteria that are involved in the oxidation of ammonium to nitrite. If effective herbs are identified, they can be applied along with fertilizers so that the N-use efficiency and yield of crops will be improved, it will be easier to produce them locally and supply to the users at a cheap price. Therefore, this experiment was conducted to investigate the nitrification inhibition ability of Ethiopian medicinal herbs along with commercial inhibitors such as N-serve and Dicyandiamide.

Objective

Over all Objective

To increase the nitrogen use efficiency of crops and there by increase the yield by the use of nitrification inhibitors identified from Ethiopian medicinal herbs and commercial inhibitors.

Specific Objectives

1.To investigate the nitrification potential of some soils of southern Ethiopia and associated factors.

2. To study the nitrification inhibition ability of some Ethiopian medicinal herbs.

3. To study the effect of commercial inhibitors along with Ethiopian medicinal herbs on N-use efficiency and yield of wheat.

LITERATURE REVIEW

1. Importance of nitrification in nitrogen dynamics and its implication in agriculture and environment.

Plants cannot use the inert form of nitrogen from the atmosphere. They need fixed forms for their growth and development. There are various forms of fixation through which nitrogen is supplied to the soil then to living organisms. These include biological and industrial fixation and deposition from the atmosphere by electrical discharge. As there are various forms of fixation, so there is a release of elemental nitrogen to the atmosphere. And the inert nitrogen is in dynamic equilibrium with fixed ones. The transition of nitrogen from fixed to elemental and vice -versa occurs in a cycle, hence known as N-cycle. The N-cycle is crucial to ensure the continuity of life on the earth. The events in N-cycle are fixation, mineralization, immobilization, nitrification and denitrification (Tisdale *el al*, 1985; Bardy and Well, 2002).

Nitrification is a biological conversion of ammonium to nitrate. In the process ammonium is oxidized to nitrite and further to nitrate. This stepwise dissimilation of ammonium is brought about by ammonium and nitrite oxidizing bacteria respectively (Alexander, 1977). Nitrification is a key process in N-cycle because it is central to flow loss or utilization of N through the conversion of ammonium in to labile nitrate (Jarvis *et al*., 1996; Gregory, 1998).

Nitrification is important in agriculture and environment. Because nitrate, which is the product of nitrification, is one of the form of N taken up by plants or crops (Alexander, 1965; Coulbe *el al*., 1996). Basically, plants absorb N in the ionic form of ammonium and nitrate. However, they differ in their preferential uptake of the two forms based on their age and environmental factors. Such as cereals, corn potatoes sugar beets, pineapple, rice, and ryegrass use either form of nitrogen. Tomatoes, kale, bush bean squash, and tobacco grow best when provided with nitrate. Some plants like blue berries, chenopodium and certain rice cultivars cannot tolerate

nitrate (Tisdale *et al.* 1985).

Nitrate is the predominant source of nitrogen to plants since it generally occurs in higher concentration than ammonium and it is free to move to the roots by mass flow and diffusion (Sumner, 1999), with the exception of rice in which the major source of N is ammonium as the condition for nitrate formation is unfavorable in the rice field due to saturation of soils with water (Prasad and Power, 1997). Nitrification varies from soil to soil depending on several factors. From crop production point of view, in soils where the rate of nitrate release is synchronized with crop demand has an advantageous and important as the nitrogen coming from mineralization of organic or inorganic sources or both will be utilized efficiently (Campbell *et al*, 1995). On the other hand if nitrification occurs at high rate, nitrate will be produced in excess amount. In such, case the nitrate produced may be leached or the N- may be lost through denitrification (Gregory, 1998). The net effect of which is reduced N-use efficiency and yield of crops. This will ultimately result in economic loss. Most soil microorganisms grow best with ammonium than Nitrate. Excess amount of nitrate in the soil adversely affects the growth and proliferation of soil microorganisms. This is because most soil organisms lack the enzyme, nitrate reducase responsible for assimilation and denitrification of nitrate. Even for those soil microorganisms endowed with the ability to grow with nitrate, there is a need for the supply of high amount of carbon source. Because the assimilation of nitrate or its conversion to protein in the body of microorganisms requires high amount of energy (Azam and Farooq, 2003). Thus, maintenance of N in the form of ammonium enhances the growth of soil microorganisms and mineralization of native soil N (Jenkinson *et al*., 1985).

From environmental point of view, if high amount of nitrate is produced, it will be leached out of the soil and cause pollution of ground water and that could become a threat to the health of human and animal (Armstrong and Bert, 1993; Coulobe *et al*., 1995). Currently, the issue of nitrate pollution of ground water is becoming a serious issue in developed country where inorganic fertilizers are applied in high amount in agricultural lands. In addition to leaching of nitrate and pollution of ground water,

application of NH_4^+ or NH_4^+-forming fertilizers in agricultural lands and their subsequent, nitrification causing an emission of potent green house gases to such as NO and N_2O (Breitenbeck *et al*., 1980; Estavillo *et al*., 1996; Flessa *et al*., 1996). These gases are known to deplete stratospheric ozone (Prasad and Power, 1995). Thus, the regulation of nitrate productions in the environment through the judicious use of nitrogen containing materials is gaining momentum more than ever before.

When it comes to sewage treatment that is discharged out of cities, using bioreactors, harnessing of nitrification rate is very important. As the nitrogen in urban wastes is in ammonia form and its diffusion to atmosphere causes toxicity and nuisance. Thus, harnessing nitrification followed by denirification becomes important (Pochana, 1999; Sumner, 1999).

2. Nitrifying microorganisms and their diversity.

Authotrophic or chemolitotrophic bacteria bring about the majority of nitrification (Azam and Farooq, 2003). Bacteria in this group derive energy through the oxidation of NH_4 to$^+$ NO_2 further to NO_3^-. The former ones are collectively known as ammonia oxidizers and those that oxidize NO_2 to NO_3^- are called nitrite oxidizers. In most occasions both bacteria coexist and the nitrite formed by ammonium oxidizing bacteria is rapidly oxidized to nitrate. However, in some case nitrite is accumulated in soils with high level of ammonium salt (Smith, 1976) and highly alkaline soils and causes the toxicity to crops (Alexader, 1977) and high ammonium level in the soil. All authotrophic nitrifiers are belonging to family Nitrobacteriaceae. Currently, only five genera of ammonia oxidizers whose name beginning with Niroso and four genera of nitrite oxidizers whose name beginning with Nitro are known (Coyne, 1999: Sumner, 1999). The five genera of ammonium oxidizing bacteria known to date are *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio*. However, *Nirosomonas* is the most frequently encountered and identified genus. The four genera of nitrite oxidizing bacteria known to date. These are *Nitrobacter*, *Nitrospirina*, *Nitrospina* and *Nitrococcus*. Nitrifying bacteria are expected to be more divers in terms of kind and ecology. And yet to what extent they

are divers is not fully discovered. This is mainly because in depth study of nitrifying bacteria is limited by them fact that they are difficult to isolate the in pure culture in contrast to most other bacteria (Broke and Madigan, 1994).

An attempt to isolate nitrifying bacteria usually fails, as they are too sensitive to small amount of organic matter present in the medium. Besides, they are too slow growers or have a very long generation time. As a result the medium is contaminated by heterothrophs making isolation complicated (Schimidt and Bleser, 1994; Bruce *et al*.; 1996). However, with the advent of molecular techniques more and more information on the diversity of nitrifying bacteria is likely to be obtained (Degrange, *et al*., 1998). This days molecular techniques are employed not only to study the taxonomy of nitrifying bacteria but also to enumerate and determine there population size of nitrifiers in a particular soil sample (Hermansson, *et al*., 2004).

Although, authotrophic bacteria accomplish most nitrification processes, there are also heterotrphic microorganisms that can catalyze the conversion of ammonium to nitrate. Heterotrophic nitrification occurs in fungi, actinomycetes and bacteria (Prosser, 1989). Keeney (1980) reported that in acid forest soils authotrophic nitrification is limited by pH and high amount of organic matter in the profile. In their study, Kreiting *et al*., (1985) found that when a soil sample obtained from acid soils incubated with the addition of NH_4^+ no enhancement in NO_3^- formation was noticed. But when the same soil was incubated with amendment of peptone an appreciable increase in the production of nitrate was obtained. Their finding indicated that in these soils agents responsible for nitrification were methylotrophs type of organisms. Some genera and species of heterotrophic microorganisms identified to catalyze the conversion of ammonium to nitrate are fungi (*Aspergillus flavus, Neurospora crossa, Penicillium sp*.), actinomycetes (*Strepotomyces sp., Nocardia sp*.) and bacteria (*Athrobacter sp., Nocardia sp. Pseudomonas flourescens , aerobacter aerogenes, Bavillus megaterium, Proteus sp*.)

Even though, there are may evidences for the presence of heterothrophic nitrification, the argument forwarded by these authors seems to be disproved by the

finding of De Boer *et al*.; (1990). These authors reported that nitrification in the Dutch heathland soils is mediated by acid tolerant pH dependent and independent chemolithotrophic organisms.

3. Biochemistry of nitrification

Like any authotrophic bacteria, nitrifiers are characterized by their ability to utilize inorganic compounds (ammonia and nitrite) as source energy to fix CO_2 and convert to organic form. Ammonia oxidizers produce 65 Kcal for every mole of NH_3 oxidized as shown in the reaction equation below (Broke and Madigan, 1994).

1. $NH_3 + O_2 + NADH + H^+ \longrightarrow NH_2OH + H_2O + NAD$

2. $NH_2OH + O_2 \longrightarrow NO_2 + H_2O + H+$

Sum: $NH_3 + 2O_2 + NADH \longrightarrow NO_2 + 2H_2O + NAD$

G^O = -65 kcalmole^{-1}

Nitrifying bacteria produce –18 kcal for every mole of NO_2 oxidized according to the following reaction equation.

$NO_2 + O_2 \longrightarrow NO_3$

G^O = -18 kcalmole^{-1}

During the oxidation of ammonia to nitrite two types of enzymes are involved. The first product of ammonia is hydroxyleamine (NH_2OH), catalyzed by an enzyme called ammonia monooxygenase. Subsequent oxidation of hydroxylamine to nitrite is brought about by an enzyme called hydroxylamine axidoreductase. The oxidation of nitrite to nitrate is brought about by an enzyme called nitrite oxidoreductase.

4. Factors affecting nitrification

4.1 Population of nitrifying bacteria

The primary actors of nitrification process are nitrifying bacteria. Thus, they must be present in the soil in appreciable size for nitrification to proceed. Even under similar environmental conditions, soils may vary in their nitrification potential. This is

due to variation in the diversity and population of nitrifying bacteria (Morill and Dawson, 1967). Johnson and Edwards (1979) Cited in Schimidt and Bleser (1994), experimentally reported that in Tennesse tulip-popular forest soil that low initial population sizes of nitrifiers were probably the most important factor responsible for the low nitrification rate observed. Belser (1979) and Jha *et al*., (1996) reported that the number of free living cells of ammonia and nitrite oxidizing bacteria were significantly related to nitrification.

4.2. Substrate availability

The sole substrate for energy production of nitrifying bacteria is ammonium and it is one of the most important factors affecting nitrification (Knowles, 1999). Thus, ammonium coming from mineralization of organic or inorganic source must be present for nitrification to proceed. Factors that reduce the availability of ammonium such as ammonia volatilization, and immobilization reduce nitrification (Prasad and Power, 1997). Increasing the concentration of ammonium was reported to stimulate nitrification. Hayatsu and Kosuge (1993) reported that increasing the concentration of ammonium from 20 $mg100g^{-1}$ to 200 $mg100g^{-1}$ of soil has steadily increased nitrification. However, they did observe that increasing the concentration of ammonium beyond 200 mg decreased nitrification rate. Similarly, Kreitinger *et al*., (1985) reported that nitrate formation was stimulated when 47 $mgml^{-1}$ of NH_4^+ was added. But it was depressed at a concentration of 94 $mgml^{-1}$. This is because excess supply of ammonia causes toxicity to nitrifying bacteria (Brady and Well, 2002). Mahli and McGill (1982) studied the effect of increasing concentration of 50, 100 and 300 μgg^{-1} NH_4^+ -N g^{-1} of soil. They found that nitrification rate increased steadily up to 200 μgg^{-1} NH_4^+-N g^{-1} of added salt. But it was depressed at 300 μgg^{-1} of soil. The suggested probable reason for this depression was the combined adverse effect of decreased in pH and increasing in salt content from 200 to 300 μgg^{-1}.

4.3. Soil moisture and Temperature

Nitrifying bacteria are highly sensitive to soil moisture than most other authotrophic bacteria. The sensitivity of nitrifiers to soil moisture is related to their high energy requirement diverting energy source which might otherwise be used to synthesize compatible solute, such as amine or polyols, which could help them to withstand dry condition (Sprent, 1987; Brady and Well, 2002). Desiccation reduces nitrification rate drastically. A linear decline of nitrification rate was observed by Glimour (984) when the soil moisture decreased from 0.2-.12 gg^{-1} (-40 to –140 Pka). Nitrification increase with increasing soil moisture content (Morill and Dawson (1967). But when the soil water content exceeds beyond field capacity, decline in nitrification occurs. This is due to the fact that the pores will be filled up with water as a result the bacteria will be deprived of oxygen (Tate, 2000; Breuer *el al*.2002).

In the field, the oxidation of ammonium takes place up to 60^0C down to freezing point. However, the oxidation of nitrite to nitrate has been shown to be stopped at 40^0C (Tate, 1999). The same author has indicated that the optimum temperature for nitrification to proceed rapidly is between 30 and 35^0C. Nitrifiers present in tropical soils are found to be more tolerant to high temperature than those in the temperate (Myers, 1975). Justice and Smith (1962) suggested that the most ambient temperature for nitrification to occur rapidly in the laboratory incubation experiment is 25^0C. Similarly, Schimdit and Belser (1994) suggested the optimum temperature for nitrification in the laboratory incubation to be 25^0C.

4.4. Soil reaction (pH)

Initial soil pH as a major factor affecting nitrification rate of soils have been reported by several authors (Alexander, 1977; Fotch and Verstraete, 1977: Paul and Clark, 1989). Because it affects the availability of essential nutrients and functioning of enzymes proteins and nucleic acid (Edwards, 1990). According to Coyne (1999) nitrification is most rapid in neutral to alkaline soils. Glimour (1984) reported that nitrification increases linearly with initial soil pH over the range of 4.0 to 7.7. In a similar study, Hayatsu and Kosuge (1993) found that the optimum initial soil pH for to proceed rapidly nitrification to proceed is between 7 to 9. Still there some genera of

ammonium oxidizing bacteria that can perform even at pH value of as high as 9.5 (Coyne, 1999). One possible mechanism employed by microorganism growing in alkaline condition is sodium antiport method. The details of some of the possible mechanism found in alkalophilic organisms are described in Edwards (1999). Nitrification by itself is an acid producing process thus the effect of high pH could be lowered by H^+ ions produced during nitrification (Prasad and Power, 1997). According to Keeney (1980) authotrophic nitrification is negligible in acid soils and it doesn't occur below pH of 5.0 (Coyne, 1999). The occurrence of nitrification in acid soil is mediated by tropic microorganisms (Krieting *et al*, 1985). However, recent heterotrophic studies in the Dutch heath land soils by De Boer *et al*., (1990) indicated that there are acid tolerant authotrophic nitrifying bacteria that can perform nitrification at pH value as low as 4.0.

4.5. Aeration

Nitrifying bacteria are obligate authotrophic aerobes, thus the supply of oxygen is critical for their activity (Alexander, 1977; Coyne, 1999). There will not be any nitrate production in the absence of molecular oxygen. Instance of this occurs in soils where all soil pores are occupied by water. Soil that are coarse textured and having a good structure facilitate rapid exchange of gases and ensure adequate supply of oxygen for nitrifying bacteria (Prasad and Power, 1997).

4.6. Plant community and mineral factors

Other factors that affect nitrification include soil structure, texture, C/N ratio nutrients like Cu and P proper balance of Cu, Mn, Fe, and others, In general the magnitude of interaction of factors determine the extent of nitrification (Trolestra *et al*., 1990; Ste-Marie and Pare, 1999).

The wider C/N ratio slows down the nitrification process in the soil. This mostly happens when the crop residues with wide C/N ratio are added to the soil freshly (Prasad and Power, 1997). This due to the fact that non-nitrifying

heterotrophic microorganisms become active and utilize the available inorganic N to decompose the residues leading to immobilization of N. As the result the substrate of nitrification which is ammonium becomes limiting (Brady and Well, 2002).

Different ecosystems have different nitrification potential. In arable and other tillage systems nitrate production is very high as a result ammonium accumulation is rare. That is the rate of nitrification exceeds mineralization (Jarvis *et al*, 1996). Where as in grassland and in most forest soils, nitrate production is very small and significant quantities of ammonium is accumulated in the profile (Jarvis and Barraclough, 1991). The reasons for low nitrate production in the forest grass land have been the subject of controversies for many years. Some scientists say that high nitrification rate un climax forest soils is due to low availability of $NH4^+$ or uptake of NO_3^--N soon after its production not by the inhibition by root exudates(Vitoousek and Reiners, 1975; Vitousek, 1977; Johnson and Edwards, 1979). On the contrary, Theron (1951) reported that the low NH_4^+/NO_3^--N ratio in soils where some vascular plants are dominant is due to the inhibition of nitrification by the root exudates these plants. This idea was substantiated by the finding of Rice and Pancloy (1972) who reported that nitrification inhibition increases with succession. Currently more and more evidences are accumulating towards the later concept (Rice and Panchloy, 1973; Rice and Panchloy, 1974; Lodhi, 1978; Paavolianen *et. al*. 1998).

5.0 Nitrogen Use efficiency

Nitrogen is the most limiting nutrients in most soils and it represents one of the highest inputs cost in agricultural system (Thomason *et al*., 2002). Because of this fact there is an increasing concern to increase N-use efficiency. There are several ways of expressing N-use efficiency. From agronomic perspective, nitrogen use efficiency (NUE) is defined as grain production per unit of N-available in the soil and calculated as grain weight divided by N-supplied (GW/Ns) (Moll *et al*., 1982). N-uptake efficiency or apparent recover (ANR) is calculated as the difference between N-uptake in treated plot and N-uptake in 0-chek divided by total N-supplied (Thomason *et al*., 2002). There are a number of factors affecting NUE and ANR.

Such factors include soil type, climate, and growth stage of plant, variety, type of fertilizers and methods and time of application (Nova and Loomis, 1981). Knowledge about these factors helps to reduce N loss and improve economic return (Zemenchik and Albecht, 2002).

There are several methods of increasing N-use efficiency. Some of them are proper time and method of application of fertilizers, use of slow release or controlled release fertilizers (Shaviv, 2001) and application of nitrification inhibitors (SubbaRao, 1993).

6.0 Nitrification Inhibitors

Nitrification inhibitors are chemical compounds that kill nitrifying microorganisms or interfere with their metabolism (Hauck, 1984). According to Nelson and Huber (2001) nitrification inhibitors (NI) are chemical compounds that reduce the rate at which ammonium is converted to nitrate by killing or interfering with metabolic activity of ammonium oxidizing bacteria. Nitrification inhibitors were first introduced to commercial market in 1976. N-serve is the first NI discovered and subsequently more than a dozens of NI compounds were developed (Prasad and Power, 1995). There are different mechanisms through which nitrification inhibitors block or inhibit oxidation of ammonium to nitrite.

These involves inhibiting the growth of nitrifying bacteria by creating unfavorable microenvironments, by stimulating the growth of competitive microorganisms and otherwise changing the cell structure or by interfering with reductive assimilation of carbon dioxide. Some of agriculturally important NI such as N-serve inhibits ammonium oxidation by chelating with copper ion component of cytochrome oxidase enzyme of ammonium oxidizing bacteria (Hauck, 1980).

6.1. Nitrification inhibitors and their benefit to crop production

Nitrogen is one of the most important nutrients required by plants for their

growth and development. In nature the majority of it is supplied to plants through biological nitrogen fixation and little from atmospheric deposition in the form of NO_2 and NO formed by thunder storm (Tisdale, *et al,* 1985). The nitrogen fixed by legumes in symbiotic association with microorganisms or that fixed by free living fixers could be utilized by non fixing crops when the legumes and microorganisms die and their tissue decompose and release available form nitrogen. Thus the growing of cereal crops in rotation with legumes, application of manure, compost, fallowing and incorporation of crop residues have been traditional practices of supplying nitrogen to growing crops for increased yield.

The increasing demand for more food to feed the ever increasing world's population led to the synthesis and application of inorganic N-fertilizers9 Shaviv, 2001). Currently, it is true that the world's food and industrial crop production depends most on inorganic fertilizers. Nitrogen is the most limiting nutrients in the world and hence applied in greater quantities as chemical fertilizers (Bockman and Olfs, 1998). In USA alone, the consumption of N-fertilizers increased from 956,000 metric tons in 1949 to 11079,000 in 1982 (Tisdale *et. al*., 1985). Similarly the world fertilizer consumption has grown from 36 million mt in 1972 to 93 million mt in 1982 (Englestand, 1985). Recent statistics by FAO (2001) indicates that 42 million tons of fertilizer N is being applied annually for the production of 3 major cereals crops (wheat, rice and maize). At the same time the cost of fertilizers are soaring up becoming unaffordable to most farmers, especially to those living in developing countries (Sahrawat and Mukerejee, 1977).

To make a benefit out of the application this expensive material increasing the fertilizer use efficiency of crops is very important. This because following the application of nitrogenous material especially inorganic fertilizers, transformation takes place rapidly, hence leads to loss of nitrogen. According to (Hoeft, 1984) of all nutrients, N is the one that has the greatest potential to be lost from the soil system. For instance, in Ohio soils not more than 50% of the applied N is utilized by plants, the rest is lost through leaching and denitrification (Nelson and Hubner 2001). In the tropics it was found that only 25-40% of the applied Fertilizers are used by plants (

Sahrawat *et al*.,1977).

The major avenues of N-loss from the soil system is leaching and denitrification following nitrification (Woldendrop and Laanbroek, 1989; Jarvis *et.al.*,1996). Nitrification is a key process in N-dynamics as it supplies NO_3 which is one of the forms N taken up by plants (Gregory, 1998). However, when nitrate is produced in amount excess of plant demand it will be lost through leaching and denitrification. The net effect of excess nitrification is reduced fertilizer use efficiency by crops, ultimately causing economic loss and pollution.

Increasing the fertilizer use efficiency of crops by reducing the loss of N is the over all objective of fertilizer research (Reynolds *et al*., 1994). Any increase in this efficiency would mean increasing agronomic and economic value of fertilizers as a means of increasing crop production, conserve energy and raw materials needed to make N-fertilizers and maintain healthy environment.

One of the most popular strategies to increase the fertilizer use efficiency of crops is to inhibit nitrification-using inhibitors (Meisinger *et al*, 1980; SubbaRao, 1993; Prasad and Power, 1995). There are several reports indicating that nitrification inhibitors increase the yield and N-use efficiency of crops (Moore and Smiciklas, 1997; Nelson and Huber, 2001; Prasad and Power, 1995; Sahrawat, 1989). Malhi and Nyborg (1982) reported that the application of aqua NH_3 or urea plus inhibitor increased the yield of barely from 800 $kgha^{-1}$ to 1370 $kgha^{-1}$. In an experiment conducted in Thailand, the effect of three inhibitors namely dicyandiamide, thiourea and sulphatiazole along with the control were studied on the yield and N-recovery by paddy rice. The result revealed that the percentage N-recovery increased from 30.33% (control or chemical fertilizers alone) to 36.7, 40.33 and 44.33% for treatments of DCD, thiourea and sulphantiazole respectively. Similarly, the grain yield was highest for treatments that received fertilizers with inhibitors (Chaichuary, 1993). Appreciable increase in the yield and N-uptake of corn has been reported in USA (Moore and Smiciklas, 1997; Johnson, 2002). Bock (1987) compared the effect of basal application of NO_3^--N and Urea + N-serve NO_3^--N alone and he observed a 19 to 45% increases in the yield of wheat. In a similar study, Camberto and Bock (1989) reported

a 15 to 18% increase in the grain yield of sorghum when high concentration of NH_4^+-N from urea was maintained using N-serve. Nelson and Hubner (1980) studied the effect of anhydrous ammonia and N-serve applied to corn for four years (1973 – 1978), they found that yield the could be increased by 24% over four years. According to International Rice Commission, ammonium sulphate treated with treated N-serve increased the yield of rice by 15-20% over untreated ammonium sulphate. Ownes (1987) found that the annual loss of N from the application of 336 $kgha^{-1}$ in the form of urea to corn planted to Rhyne silt loam soil (Typic hapludult) was 160 $kgha^{-1}$. But this loss has been reduced to 117 $kgha^{-1}$ when the same amount of N was treated with 1.12 $kgha^{-1}$ N-serve. Freney *et al* (1993) studied the effect of N-serve, acetylene (Provided by wax coated calcium carbide), phenyl acetylene on nitrogen recovery and lint yield of irrigated cotton in Typic pellustret soil of Australia using ^{15}N labeled fertilizer. They found that the N-recovery in the plant and soil at crop maturity to be 57% in untreated control soil. The recovery was increased to 70% by the addition of phenyl acetylene to 74% by N-serve to 78% by coated calcium carbide and to 92% by 2-ethynylpyridine.

There are nitrification inhibitors of plant origin. According to Sahrawat and Mukerjee(1977) none edible oil seed cakes of karanaja (*Pongamia glabra*) and neem (*Azadirachta indica*) have been used since time immemorial in admixture with manure. Name cake-coated urea on different soils (Entisol, Vertisol and Ultisols) was compared with as N-serve for its effectiveness in conserving NH_4^+-N. It was found that neem cake was found to be 50% as effective as N-serve (Thomas and Prasad, 1982). The beneficial effect of these cakes was attributed to their inhibitory effect on nitrification. The active compounds in neem resposonsible for retardation of nitrification are thought to be meliacins (epinibin,nimbin, deacetyl nimbin, salanin desacetylsalanin and azadiractin) (Devacumar, 1986). Sahrawat *et al*., (1977) compared N-serve and karanjin, a major crystalline precipitate of karanja (*Pongania glabra* vet), which is a potent inhibitor of nitrification. And they found that both inhibitors significantly increased dry matter production and N-uptake in rice. The same authors reported that Karanjin extract applied at 15% of N in the form of ammonium increase N-uptake of rice by 68% over the untreated control. Rice and

Panchloy (1974) reported that condensed tannins and their derivatives such as ellagic acid, gallic acid and digallic acid isolated from aoks and pine were found to inhibit nitrification even at a very low concentration. In a similar study Paavoainen *et. al*. (1998) found that monoterpenes that are produced by roots of Norway Spurce (*Picea albies* L.).

Even though nitrification inhibitors are beneficial in improving the yield and N-use efficiency of crops, it does not mean that their applications are always beneficial. Their benefit is limited to conditions where there is severe loss of nitrogen due to leaching and denitrification following excess nitrification (Hauck, 1984; Johnson, 2001). Nitrification inhibitors are used effectively with fertilizer compounds like anhydrous ammonia, urea ammonium nitrate, ammonium sulphate and ammonium nitrate. Since nitrification inhibitors influence the conversion of ammonium to nitrite (Hauck, 1984).

In general the potential benefit of nitrification depends on soil type, climatic condition, cultural practice crops to be grown and N-management (Moors and Smiciklas, 1997). Soils that are excessively drained or poorly drained have greater potential for N-loss than those that are well or somewhat poorly drained. Application of nitrification inhibitors on caorse textured soils are more useful than fine textured one since N-loss through leaching is more in the former than in the in the later (Hauck, 1984). It must be recognized that nitrification inhibitors are beneficial when low rates of fertilizers are applied (Vitosh and Jacobs, 1996).

6.2. Important characteristics of Nitrification Inhibitors

Any compound in addition to inhibition of nitrification it has to be non toxic to plants, or other organisms, block the conversion ammonium to nitrate by specifically inhibiting ammonium oxidizers, not interfere with the transformation of nitrite by nitrite oxidizers (Tate, 2000), be able to move with fertilizers or fertilizer solution, be stable enough for its inhibitory action to last for an adequate period of time and be relatively inexpensive so that it can be used on a commercial scale (Hauck,1984).

7. 0. Factors affecting effectiveness of Nitrification Inhibitors

Effectiveness of nitrification inhibitors varies from soil to soil. For instance, McCarty and Bremner (1989) compared 11 compounds on three soils of USA. They found that six of the inhibitors to be effective in general and highest percentage inhibition of nitrification was achieved in Stroden soil followed by Webster and the least in Harpen soil (Table 1). There are several factors affecting the effectiveness of nitrification inhibitors. The most prominent factors are organic matter, temperature, soil moisture content, form of N, management (Meisinger, *et al*., 1980; Prasad and Power, 1995).

Table 1. Effect of 5 mg kg^{-1} soil with different compounds on nitrification of Ammonium in soil.

Compounds	**Soil**		
	Harps	**Webster**	**Stroden**
	% Inhibition of nitrification		
2-Ethynpyridine	79	80	100
Etridiazole(Dwell)	61	70	97
Nitripyrin(N-serve	45	56	94
3-methylpyrazole-1-carboxamide	43	53	93
4-amino1,2,3-triazole	41	52	92
Dicyandiamide	8	20	41
N(2,5-Dichlorophenyl)succinamide	0	3	5
Sodium thiocarbonate	0	2	5
Thiourea	0	0	0
2-Mercaptobenzothiazole	0	0	0
Ammonium thiosulphate	0	0	0

Source: McCarty and Bremner (1989)

7.1. Organic matter content of a soil

Organic matter (OM) content of a soil is the most prominent factors that affect the effectiveness of nitrification inhibitors. Hendrickson and Keeney (1979) in their experiment increased the organic matter of sandy loam soil originally having 1% OM to 2 and 5% by adding peat together with N-serve and $(NH_4)_2HPO_4$, they found that N-serve was more effective in untreated soil with OM. This is due to the sorption of N-serve by OM (Prasad and Power, 1995). Despite, the general finding that increase in Om result in decreased effectiveness of inhibitors, there is an exception where OM content is counter acted by other factors such as pH and texture. For instance, Touchton *et al*. (1978) found that N-serve was more effective in controlling nitrification in silty clay loam soil with 5% OM than in silty soil with less than 2%.

7.2. Temperature

In most studies conducted it was found that nitrification inhibitors perform best below temperature of 20^0C, which is well below laboratory temperature (Goring, 1962, Bundy and Bremner, 1973). This is due to the greater persistence of inhibitors as a result of slow degradation, low volatilization and slow nitrification rate at low temperature. Herlihy and Quirk (1975) compared the half-life of N-serve at two temperature points. They found that the half-life N-serve to be 22 days at 4^0C and less than 13 days at 21^0C. Inhibitors vary in their sensitivity to high temperature. For example, DCD is very sensitive to high temperature than others. Vilsmeier (1980) reported that after 60 days, 0.67mg DCD 100 g^{-1} soil degraded to 0.6 mg at 8^0C, 0.4mg at 14^0C and 0.1 mg at 21^0C in a sandy silt loam soil of Germany with pH of 6.2

7.3. Form of N

Nitrogen fertilizers are broadly categorized as alkaline forming fertilizers, such as urea and NH_3 and acid forming ones such as $(NH_4)_2SO_4$, NHN_4O_3 and $(NH_4)_2HPO_4$. Alkaline forming forms increase the pH, as a result the result

nitrification rate proceeds at faster rate. On the other hand, acid forming N forms decrease the pH leading to reduced nitrification rate. Thus, nitrification inhibitors are more effective with acid forming N-fertilizers form than alkaline forming ones.

7.4. Soil water

Water saturated soil promotes the hydrolysis of some inhibitors. Soil moisture content at field capacity is the best for the effectiveness of inhibitors (Hendrickson and Keeney, 1975). N-serve is more volatile in wet than dry condition.

7.5. Management of inhibitors and fertilizer

Management techniques such as time and methods of application have a bearing on the effectiveness of inhibitors (Meisinger *et al*., 1980). Time of application interacts with other factors such as moisture and temperature and that low temperature enhances effectiveness of inhibitors. Surface application reduces effectiveness of inhibitors due to photolysis, volatilization loss and minimal penetration in to the soil (Briggs, 1975). More inhibitor will be will be required with broad cast incorporated than with band applied N-fertilizer (Turner and Goring, 1966).

8.0. General Characteristics of some of the Ethiopian traditional medicinal Herbs used in this study.

Ethiopia is rich in its traditional medicinal herbs (Amare, 1976) and the greatest proportion of the population depends on herbs to be cured from a variety of diseases and ailments like any other developing countries. Recent scientific studies are coming up with the result proving the traditional medicinal herbs to be effective against human disease causing pathogens and parasites (Mintesinot, 1999). General characteristics of some of the Ethiopian medicinal herbs studied in this research are highlighted below.

8.1. *Artemisia Afra*

It belongs to family compositae (Hedeg and Edwards). The plant is shrubby her found in the high altitude area and grown around homesteads. He herb is used traditionally to relieve stomach pains. The crushed leaves is mixed with water or honey and administered orally. It is also used clean milk containers perhaps because of pleasant odor (Amare, 1976).

8.2. *Cymbogon citrates*

It is a perennial densely tufted cultivated grass (Kotbe, 1985). Often tall and robust. It is grown for its aromatic essential oils.

8.3. *Vernonia amigdalina*

A very common shrub tree growing in sub humid or highlands in an altitude ranging between 1000 to 2700m asl. It some attains a height above 10m (Azene *et al*., 1993). The sap is used a purgative. The dried flower is used to treat stomach disorder (Amare, 1976).

8.4. *Croton macrostachyus*

A deciduous tree, crow rounded light and open slender trunk and spreading branches reaching up to 25 heights. It grows between 110 to 2500 masl.

8.5. *Ruta chaelepensis*

It is indigenous to Mediterranean area, the Canary island, Arabic and Somali. It is cultivated all over the world in many countries. In Ethiopia it is grown around homestead. The plant is an erect perennial herb becoming woody at the base and grows up to 1.5 m high (Jansen, 1981).

8.6. ***Thumus serpyllum***

A small herb growing in the high altitude areas. It is useful condiment. The herb is also reportedly used to control gonorrhea. When it is added to boiling water and drunk, it is used against cough and liver diseases. In some provinces of Ethiopia, it is used to flavor tea or drunk alone as tea.

8.7. ***Hagenia abyssinica***

It is a big tree typically adapted to growing in the highlands above 2000 m. The dried pistillate flowers of the tree are the most widely used as tape worm expellant.

8.8. ***Echinops spp*.**

Echinops spp. Are belonging to the family compositae. They are smallherbs rarely arborescent with generally alternate, more rarely opposite, extipulant leaves (Rendle, 1979), the leaves and stems are spiny, thistle like, flower head spherical (Polunin and Huxley, 1965).

8.9. ***Eugenia caryophyllata***

Clove, small, reddish-brown flower bud of the tropical evergreen tree. It can grow from 8 to 12 m height. Its gland-dotted leaves are small, simple and opposite. The clove oil is used to prepare microscopic slides for viewing and is also a local anesthetic for toothaches. It is a strong antiseptic and preservative. It is used to treat flatulence, colic, indigestion and nausea. Eugenol is used in germicides, perfumes and mouthwashes, in the synthesis of vanillin, and as a sweetener or intensifier (http://www.harvestfields.ca/CookBooks/spice/cloves.htm).

8.10. ***Zingeber officinale***

It is a common species and its rhizome is used in the preparation of traditionali food. It is generally recognized as a medicine for stomach cramps (Amare, 1976).

MATERIALS AND METHODS

2. 1. Experiment 1. Investigation on the nitrification potential of some soils Occurring in central and southern Ethiopia

2.1. Site characteristics

Soil samples were collected from eight sites representing some parts of southern and central Ethiopia. These areas were selected based on the fact that the soils are diverse and represent the majority of Ethiopian soils. The climatic characteristics, geographical locations and soil taxonomy according to FAO/UNESCO scheme of sampling sites are presented in Table 2. The exact locations of sampling sites are shown in Figure. 1.

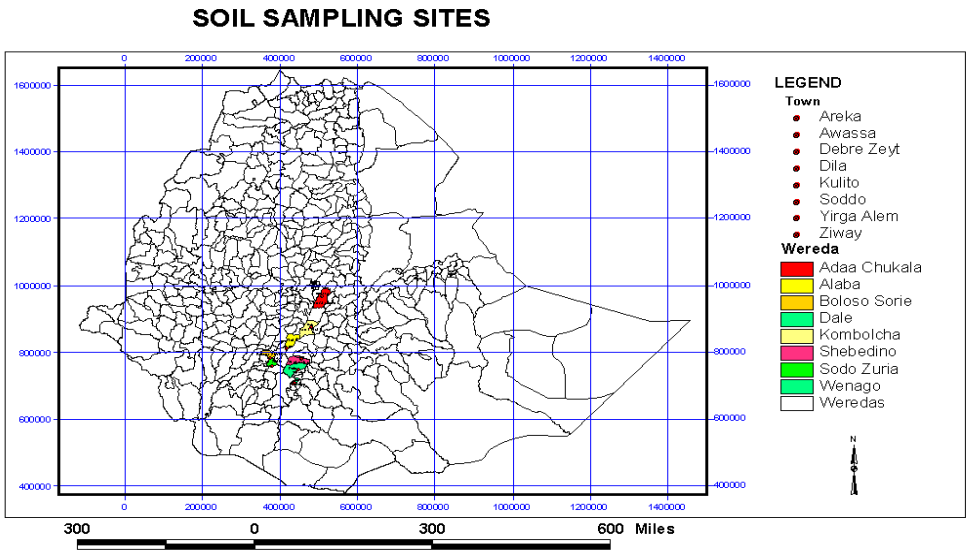


Figure 1. Ethiopian Map showing the soil-sampling site.

Table 2.The geographical position, climate and soil taxonomy of the sampling area.

	Locations/Sites							
Character	**Alaba**	**Kokate**	**Areka**	**Dilla**	**Yirgalem**	**Awassa**	**Ziway**	**Debreziet**
Latitude	$7^0$16’ N	$6^0$52’N	$7^0$03’ N	$6^0$20’N	$6^0$44’N	$7^0$3’N	$7^0$57’N	$8^0$44’N
Longitude	38^0 48’E	37^0 48’E	$37^0$41’	$38^0$16’	$38^0$23’E	$38^0$30’	$38^0$43E	$38^0$59’
Elevation (m)	1730	2156	1806	1636	1742	1844	1639	1920
Mean annual Rain Fall (mm)	990	1325	1511	1299.2	1236.2	1046	759.9	764.2
Mean Maximum Temperature (^{O}C)	20	11.5	19.95	19.3	19	19.5	20	18.2
Soil Taxonomy (FAO)	Pani-vertic Cambisol	Dystric Nitisol	Haplic Alisol	Rhodic Feralsol	Eutric Nitisol	Eutric fluvisol	Fluvi-vitric Andisol	Vitric Andisol

1.2. Soil Sampling:

Samples surface soil (0-15 cm) from 20 plots was collected using an auger. The samples were transferred in to plastic bags and brought to the National Soil Research laboratory, Addis Ababa, Ethiopia. The composite samples were thoroughly mixed in the laboratory and one kilogram of sub-samples were taken air dried, and grounded to pass 2 mm sieve and were analyzed for the following chemical characteristics: pH (1:2 soil water suspension (Mclean, 1982); organic matter (OM) by Walkley and Black's (1934) method; available phosphorus (P) by Olsen and Sommers (1982) Exchangeable bases (BS) by Thomas (1982) method; micronutrients by Lindsay and Norvell (1978). Total nitrogen(TN) and cation exchange capacity (CEC). From each site, a composite were determined using procedures described in Rowell (1994). The result soil analysis data of each site is presented in Table 3.

Table 3. Some of the physical and chemical characteristics of soil used in the Experiment

Soil Types	pH	OM (%)	TN (%)	AVP ($mgkg^{-1}$)	C/ N	BS (%)	CEC	K	Ca	Na	Mg	Fe	Mn	Zn	Cu
									Cmol kg^{-1}				$mgkg^{-1}$		
Alaba clay loam	8.0	4.2	0.129	6.3	11	76	17.2	3.44	3.66	0.37	0.99	11.5	18.3	4.08	0.10
Kokate loam	6.2	3.9	0.213	5.0	11	33	26.4	1.13	2.97	0.14	0.74	7.4	9.0	4.02	0.12
Areka clay loam	6.2	4.2	0.181	2.0	13	48	19.0	2.99	2.27	0.08	0.78	5.9	11.9	1.64	0.02
Dilla silty	6.9	4.5	0.206	3.8	13	50	34.0	1.02	5.89	0.19	1.97	6.5	11.7	1.42	0.10
Yirgalem clay loam	7.5	4.8	0.244	4.9	12	62	26.0	2.99	4.89	0.09	1.64	6.1	15.5	4.58	0.14
Awassa clay loam	7.6	3.4	0.154	46.	13	68	21.2	3.53	4.19	0.40	1.03	17.9	25.8	6.40	0.14
Ziway Ccay loam	9.1	5.08	0.239	24	9.0	97	37.0	4.15	12.55	1.36	2.71	3.1	12.7	1.48	1.34
Debreziet loam	8.8	4.8	0.183	2.8	10	79	26.2	2.43	5.25	0.46	3.58	21.5	6.9	2.26	2.84

1.3. Soil Incubation Procedure

Sub-sample from each soil was air-dried in dark and sieved to pass 4 mm sized mesh. One hundred gram (oven dry basis) of soil from each sample was transferred in 250 ml capacity plastic cups. Then the cups were amended with 2ml of $(NH_4)_2SO_4$ containing 25mg ml^{-1} of solution (Schimidt and Belser, 1994). Unameneded control cups of the respective soils were included in the experiment. The moisture contents of the soils were adjusted at 60 % water holding capacity (WHC). The cups were covered with perforated aluminum foil to allow the exchange of gases and incubated in dark at 25^0C for 3, 4, 6 weeks. At three days interval the cups were weighed and the lost water was adjusted with distilled water. The experiment comprised eight treatments (soil types) and was laid out in completely randomized design (CRD) with 3 replications.

1.4. Determination of ammonium-N and Nitrate-N

The amount of NH_4^+-N and NO_3^- -N present in the soil initially and at the end of each incubation period were analyzed following the procedure described in Keeney and Nelson (1982), in which twenty five grams of soil samples were taken and extracted with 2*M* solution of KCl (1: 5 Soil solution ratio). The extracts were filtered through Wathman filter paper N0 100. The neat filtrates were analyzed for NH_4^+-N and NO_3^- -N by steam distillation apparatus Using MgO and Devarda's alloy. The distillates were titrated against 0.01N of HCl Net production of NH_4^+-N and NO_3^- -N were calculated as difference between NH_4^+-N and NO_3^- -N measured at the end of each incubation period and those that are measured initially.

Experiment 2. Screening of Ethiopian Traditional medicinal herbs for their nitrification Inhibiting Ability

2.1. Soil sampling, preparation and analysis

A composite samples of surface top soil (0-15 cm) from 20 plots were collected using a spade. These samples were taken from a research farm of Awassa Agricultural

Research Center, Ethiopia. The samples were air dried ,and grounded to pass 2 mm sieve and were analyzed for the following chemical chracteristics: pH (1:2 soil water suspension (Mclean, 1982); organic matter (OM) by Walkley and Black's (1934) method, available phosphorus (P) by Olsen and Sommers (1982), Exchangeable bases (BS) by Thomas (1982) method and micronutrients by Lindsay and Norvell (1978). Total nitrogen (TN) and cation exchange capacity (CEC) were determined using procedures described in Rowell (1994). The soil was characterized as Awassa clay loam (Eutric Fluvisol) soil and identified as fast nitrifying soil (Wassie *et al*., 2004). Selected initial characterstics are presented in Table 4.

Table 4 Chemical characteristics of soil used in the experiment.

pH	TN (%)	OM (%)	BS (%)	Na	K	Ca	Mg	CEC	P	Fe	Mn	Zn	Cu
				cmol kg^{-1}					mg kg^{-1}				
7.6	0.15	3.4	68	0.4	5.5	4.19	1.03	21	46	18	26	6.4	0.1

2.2. Preparation and extraction of medicinal herbs

As described by Woldemichael (1987), the Ethiopian traditional medicinal herbs and Neem (None Ethiopian medicinal herb included as positive control) and their parts used in this study are listed in Table 5. These plant parts were dried in dark places and finely grounded to powder. A 100 gm of each medicinal herb was mixed with 500 ml of 95 % Ethanol (1:5 ratio) and shaked on a shaker for 48 hours at 200 rpm. The extracts were then filtered through filter paper and the alcohol was removed using rotary evaporator. The extracts were further concentrated using steam hot plate maintained at 40 ^{O}C.

Table 5 Description of some of traditional medicinal herbs screend as nitrification inhibitors (Woldemichael**,** 1987).

Common Name	**Scientific name**	**Family name**	**Parts use**
Wormwood	*Artemisia afra*	Compositae	Leaf
Lemmon grass	*Cymbopogon citratus*	Poaceae (Graminae)	Leaf
Bitter leaf	*Vernonia amygdalina* Del.	Aseraceae	Leaf
-	*Croton macrostachyus* Hochst.	Euphorbiaceae	Leaf
Herb of grace	*Ruta chalepensis* L.	Rutaceae	Fruit
-	*Thymus serpyllum*	Lamiaceae	Leaf
-	*Haginia abyssinica* Bruce.	Rosaceae	Flower
-	*Echinops* spp.	Compositae	Tap root
Glove tree	*Eugenia caryphyllata* Thunb.	Myrtaceae	Fruit
Ginger	*Zingeber officinale*	Zingeberaceae	Rhizome
Neem	*Azadiracta indica*	Meliaceae	Seed

Figure 2. *Artemisia afra*

Figure 3. *Cymbopogon citrates*

Figure 4. *Vernonia amigdalina*

Figure 5. *Croton mcrostachyus*

Figure 6. *Ruta chalaepensis*

Figure 7. *Thymus serpyllum*

Figure 8. *Hagenia abyssinica*

Figue 9. *Echinops spp.*

Figure 10. *Eugenia cayiophyllata*

Figure 11. *Zingeber officinalae*

2.3. Preliminary screening procedure.

Plastic cups containing 100 gm of processed soil were amended with 50 mg of ammonium sulphate (Substrate for nitrifying bacteria) in the form of solution. Alcohol extracts of each medicinal herb were added to plastic cup separately at 1% rate of dry soil mass. Commercial inhibitors, N-serve ([2-chloro-6(trichloromethyl) pyridine]) and Dicyandiamide (DCD) were also added at a rate of 20 and 100 μg /g of soil (Sahrawat, *et al*, 1987) respectively. Untreated control cups were also included in the experiment and three replicated cups were used for each treatment. The experiment was laid out in completely randomized design (CRD). The moisture content was maintained at 60 % water holding capacity (WHC) and incubated for 15 and 21 days at 25 ^{O}C. At the end of each incubation period soil samples were taken and analyzed for NH_4-N, NO_3-N and pH. The NH_4^+-N and NO_3^--N were analyzed using the procedure described in Keeney and Nelson (1982).

The percentage nitrification inhibition by each inhibitor was calculated according to the following formula described in Sahrawat (1980).

$$\% \text{ Inhibition} = 100(C-S)/C$$

Where, C is the amount of NO_3 -N in the untreated control cups and S is the amount of NO_3 -N in the treated cups.

2.4. Secondary screening

From among Ethiopian traditional medicinal herbs tested as nitrification inhbitors in the preliminary screening experiment three herbs namely *Artemis afra, Echinops spp* and *Eugenia caryophyllata* were found suprior to the remaining others. These herbs were rescreened for confirmation uing the same procedure described in preliminary screening experiment.

Experiment 3: The nitrification inhibiting ability of Ethiopian medicinal herbs as affected by soil types

3.0. <u>Soil sampling, preparation and analysis</u>.

A composite samples of surface top soil (0-15 cm) from 20 plots were collected using a spade. These samples were taken from a research farm of Awassa Agricultural Research center and Ziway, Ethiopia. They are located between $7^0$03'N and $38^0$30'E and $7^0$57'N and $38^0$43'E respectively.The samples were air dried ,and grounded to pass 2 mm sieve and were analyzed for the following chemical chracteristicc: pH (1:2 soil water suspension (Mclean, 1982); organic matter by Walkley and Black's (1934) method; available phosphorus (P) by Olsen and sommers (1982) Exchangeable bases by Thomas (1982) method; micronutrients by Lindsay and Norvell (1978). Total Nitrogen and CEC were determined using procedures described in Rowell (1994). Both Awassa and Ziway soils have textural class of clay loam and were characterized Eutric Fluviso and Fluvi-Vitric Andisol respectively. They were identified as fast nitrifying soil (Wassie *et al*., 2004). Selected soil intial chemical characterstics are presented in Table 6. The samples were thoroughly mixed, air dried and sieved to pass 4 mm mesh. One hundred gram of processed soil was transferred in to 250ml capacity plastic cups to which 25 mg of $NH_4)_2SO_4$ was added in the form of solution. Alcohol extracts of each medicinal herb (Table 5) was added to plastic cups separately at 1% of dry soil mass. The preparations of medicinal herbs extraction procure are described in section 2. Commercial inhibitors, N-serve and DCD were added at a rate of 20 and 100 µgg-1 of soil respectively (Sahrawat *et al*., 1987)

Table 6 Chemical characteristics of Soil used in the experiment.

Soil Type	pH	TN (%)	OC (%)	BS (%)	Na	K	Ca	Mg	CEC	P	Fe	Mn	Zn	Cu
					cmol kg^{-1}					mg kg^{-1}				
Awassa Caly Loam	7.6	0.154	1.97	68	0.4	5.53	4.19	1.03	21.2	46.0	18	26	6.4	0.14
Ziway Clay Loam	9.1	0.239	2.15	97	1.36	4.15	12.55	2.72	37	24.72	3.08	12.7	1.48	1.34

A 2 x 14 factorial experiment consisting of two soil types and fourteen levels of inhibitors were laid out in CRD with 3 replications**.** The moisture content was maintained at 60% WHC and incubated for 15 and 21 days at 25^0C**.** The NH_4^+-N and NO_3^--N were analyzed using the procedure described in Keeney and Nelson (1982).

The percent nitrification inhibition by each inhibitor was calculated according to the following formula described in Sahrawat (1980).

% Inhibition = 100 (C-S)/C

Where, C is the amount of NO_3^- -N in the untreated control cups and S is the amount of NO_3^- -N in the treated cups.

4.0. Experiment 4: The effect of nitrification inhibitors on N-use efficiency and yield of Wheat

Greenhouse pot experiment was conducted based on the result obtained from experiment 1 and 2. In the first experiment the soil that has high nitrification potential with low initial soil nitrogen content was identified. This is because response to nitrogen fertilizer application and nitrification inhibitors is obtained provided that the soil has N content below critical level and also the response to nitrification inhibitors is expected when applied to soils with high rate of nitrification rate. In the second experiment Ethiopian traditional medicinal herb that are effective as nitrification inhibitor was identified. The soil was collected from Awassa Agricultural Research Center, Ethiopia located between $7^0$03'N and $38^0$30'E. The site has an altitude of 1844 masl, mean annual rainfall and temperature 1046 mm of 20.2^0C respectively. The samples were collected from 20 plots from a depth of 0-15 cm using an augur and brought to the National Soil Research laboratory, Addis Ababa where the greenhouse experiment was conducted.

4.1 Soil preparation

The soil samples were thoroughly mixed, air-dried in shade and milled to pass 4 mm sized mesh. Then 3 kg of the processed soil was filled in plastic pots of size 8 x 8 m.The soil was characterized as Eutric Fluvisol and its selected chemical and physical characteristics are shown in Table 3 section 2.1.

4.2. Experimental design and treatments

A factorial experiment consisting of 5 kinds of inhibitors (I) and 5 levels of N fertilizers (N) were laid out in RCBD design and replicated 3 times. List of treatments and their combinations are shown in Table 7. The source of N used in this experiment was ammonium sulphate. The amounts of N applied to pots were calculated per hectare basis. And different rates of N applied per pots are shown in Table 8.

Table 7. Treatment combination of greenhouse pot experiment

Inhibitors	Nitrogen ($kgha^{-1}$)				
	0 (N0)	23 (N1)	46 (N2)	92 (N3)	138 (N4)
No Inhibitor (I0)	I0-N0	I0-N1	I0-N2	I0-N3	I0-N4
Eugenia caryophyllata (I1)	I1-N0	I1-N1	I1-N2	I1-N3	I1-N4
Neem (I2)	I2-N0	I2-N1	I2-N2	I2-N3	I2-N4
N-serve (I3)	I3-N0	I3-N1	I3-N2	I3-N3	I3-N4
DCD (I4)	I4-N0	I4-N1	I4-N2	I4-N3	I4-N4

Ethanol extracts of *Eugenia carophyllata* and neem were applied at 0.1% of the soil. These herbs were selected based on their effectiveness as nitrification inhibitor in the laboratory (Wassie *et.al*., 2006). Commercial inhibitors, N-serve and DCD were applied at 0.55 and 6 kg ha^{-1} (Prasad and Power, 1995) respectively. N-serve was dissolved in acetone and mixed with the solution of ammonium sulphate in

plastic. Then the mixtures were thoroughly incorporated with soil in the pot as per the treatment.

Table 8 Different rates of N applied in the pot experiment calculated based on per hectare basis.

Nitrogen(N) Levels	N $kgha^{-1}$	$(NH_4)_2SO_4$ $kgha^{-1}$	N $mgpot^{-1}$	$(NH_4)_2SO_4$ pot^{-1}
N0	0	0	0	0
N1	23	110	53.0	253
N2	46	219	105.8	504
N3	92	438.1	210	1000
N4	138	657	317.1	1510

Wheat variety HAR 604 was plated at a rate of 150 $kgha^{-1}$ and after germination 10 plants per pot were maintained up to harvest. Basal phosphorus fertilizer in the form of triple super phosphate (TSP) was incorporated in the soil at a rate of 20 $kgha^{-1}$. The pots were regularly watered with deionized water.

4.4. Data collection.

Data on plant height, number of tillers, straw and grain yield and percent nitrogen content at booting stage and percent nitrogen content of grain and straw were taken.

4.5. Determination of nitrogen content at booting stage.

Leaf samples from each treatment near booting stage were taken and kept in paper bags. The bags were then dried in an oven at 70^0C for 48 hours. After that the dried leaves were grounded to powder. 0.1 gm of the samples were analyzed for total

nitrogen using procedure described in Rowell (1994). The nitrogen content of leaves was expressed in percent.

4.6. Harvesting of wheat and determination of straw and grain yield.

At physiological maturity, the wheat plants in each pot were collected by cutting at ground level with a sickle and were kept in a pre weighed paper bag. Then the bags containing biomass were weighed in a balance and recorded. The total weight minus the weight of the bag gave the total biomass weight. After that the grain yield were separated from the straw by hand crushing and weighed. The straw yield was determined by subtracting the grain yield from total biomass.

4.7. Determination of total nitrogen in the grain and straw

Grain and straw yield samples from each treatment were ground and 0.1grams of powder were taken and analyzed for total nitrogen according to the procedure described in Rowell (1994). The amount of N in both grain and straw were expressed in percent.

Straw N-uptake (SNU) and grain N-uptake (GNU) were determined by multiplying the percent N in each treatment with their respective straw and grain yield. Total N-uptake (TNU) was calculated as a sum of straw and grain N-uptake of each treatment.

N-use efficiency of each treatment was expressed in terms of apparent recovery efficiency (AR) (Rao and Ghai, 1986). It was calculated as follows:

Apparent recovery efficiency (AR) = (TNU in the treated pot – TNU in untreated pot or check / Rate of N applied) 100

5.0. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SAS software version 6.2 (SAS, 1989). When the ANOVA were found significant, means were separated Using Duncan multiple range test (DMRT). Correlation and regression analysis were performed as well using the same soft ware.

RESULTS AND DISCUSSION

Experiment 1: Investigation on the nitrification potential of some soils occurring in central and southern Ethiopia

1.1. Nitrification pattern under unamended condition

Nitrate nitrogen (NO^-_3-N) produced by eight mineral soils incubated under unamended condition (without the addition of $(NH_4)_2SO_4$) for 3, 4 and 6 weeks period is presented in Table 9. The soils varied significantly ($P<0.001$) in their nitrifying ability across all periods of incubation and apparently nitrification was detected in all soils. The amount of NO^-_3-N produced by the test soils at 3rd, 4th and 6th weeks of incubation period varied between 3.18 - 144.63, 4.62 - 42.58 and 9.87 - 33.32 mg kg^{-1} of soil, respectively. Our results are comparable to that reported for tropical soils (Sahrawat, 1982). At the end of 3rd week the highest amount of NO^-_3-N was accumulated by Ziway clay loam (Fluvi Vitric Andisol) soil, followed by Awassa Clay loam (Eutric Fluvisol), Yirgalem clay loam (Eutric Nitisol), Debreziet loam (Vitric Andisol) and Alaba clay loam (Plani Vertic Cambisol) soils in that order. There after there was no further increase in NO^-_3-N production with increase in time, even there was a decline in some of the soils, Particularly in Ziway clay loam soil. The site from which this soil was sampled has previously been planted to haricot bean. The possible causes are either immobilization or and denitrification by some soil bacteria. The occurrence of denitrification in aerobic soil has been reported by Azam and farooq (2003). According to these authors bacteria belong to the genus Rhizobium (N-fixing bacteria) are naturally endowed with the ability to avoid the accumulation of NO_3^- through dissimilatory reduction or denitrification, as NO_3^- is toxic to this group of bacteria. On the contrary, Kokate loam (Dystric Nitisol), Areka clay loam (Haplic Alisol) and Dilla silty soils produced the least amount of NO^-_3-N at 3rd week but there was a linear increase in accumulation of NO^-_3-N with time suggesting the rate of nitrification in these soil is relatively slow.

Table 9. Nitrate (NO_3^--N $mgkg^{-1}$) content of 8 mineal soils incubated aerobically under unamended condition with ammonium sulphate at 25^0C.

Soil Type	Incubation Period in weeks			
	Initial			
		3	4	6
Alaba clay loam	12.9	21.3d*	26.4bc	18.4d
Kokate loam	5.0	12.9c	22.4cd	38.1a
Areka clay K loam	2.3	3.2f	15.1e	22.3c
Dilla silty loam	2.0	3.5f	4.6f	9.9f
Yirgalem clay loam	16	27.43c	17.8de	13.9e
Awassa caly loam	21	46.6b	30.1b	24.8c
Ziway clay loam	27	144.6a	42.6a	33.3b
Debreziet loam	22	25.6c	23.4cd	16.9de
CV (%)	-	6.0	6.2	3.7

*Means followed within column followed by different letters are statistically different from each other according to DMRT.

1.2. Nitrification Pattern under amended condition

Nitrate- nitrogen produced by eight mineral soils amended with $(NH_4)_2SO_4$ is shown in Table 10. Accordingly, the pattern of nitrification exhibited by respective soil across all dates of incubation is similar to that observed under unamended condition. High amount of NO^-_3-N was produced by each soil under amended condition than that produced by each soil under unamended condition. This is due to the fact that $(NH_4)_2SO_4$ is the sole substrate of nitrification and its addition stimulates nitrification (Hayatsu and Kosuge, 1993; Brady and Well, 2002). The amount of NO^-_3-N produced at 3rd, 4th and 6th weeks of incubation varied between 39.5 - 180.92, 72.91- 162.46 and 92.83 - 132.46 mg kg^{-1} of soil, respectively. Still, Ziway clay loam

soil produced the highest NO^-_3-N at the end of 3rd week but there after it has declined. Alaba, Awassa, Yirgalem and Debrezietz soils produced the next highest amount of NO^-_3-N but achieved their maximum at 4 week. In the remaining soils there was a linear increase in NO^-_3-N production and achieved their maximum at the end of 6th weeks.

Table 10 Nitrate (NO_3^- -N $mgkg^{-1}$) content of 8 mineal soils incubated aerobically amended with ammonium sulphate at 25^0C.

Soil Type	Initial	Incubation Period in weeks		
		3	4	6
Alaba clay loam	12.9	143.8b*	146.8abc	132.5ab
Kokate loam	5.0	51.9e	100.6d	124.1c
Areka clay loam	2.3	43.18e	78.3e	106.4d
Dilla silty loam	2.0	39.5e	72.9e	92.8e
Yirgalem clay loam	16	113.0d	138.5ab	135.7a
Awassa caly loam	21	123.2cd	157.7ab	113.9d
Ziway clay loam	27	180.9a	162.5a	129.7abc
Debreziet loam	22	130.5bc	142.6bc	124.8bc
CV (%)	-	8.6	7.0	4.0

* Means within column followed by different letters are significantly different from each other according to DMRT

1.3. The effect of incubation period and $(NH_4)_2SO_4$ on soil NH_4^+-N content

The corresponding NH_4^+-N measured in each soil under both unamended and amended condition across all periods of incubation is presented in Table 11. Under unamended condition almost all soils had negative values of NH_4^+-N (net loss of NH_4^+ -N). This is due to the fact that the rate of ammonium oxidation to nitrate is faster than its production by mineralization (Weieler and Gilliam, 1986). This is due

to the fact that most soil temperatures are suitable for nitrification than for mineralization and as a resul the oxidation of ammonium is faster than its formation (Myers, 1975). There was also a decline in the NH_4^+-N in all soils incubated under amended condition with time. In fast nitrifying soils of Alaba, Yirgalem, Awassa, Ziway and Debreziet soils, almost all of the added NH_4^+-N has been nitrified at the end of 6th week. On the contrary, in the slow nitrifying soils of Kokate, Areka and Dilla area, substential amount of NH_4^+-N has been present at the end of 6th week.

Table 11. Ammonium-N content of 8 mineral soils incubated aerobically under ammonium sulphate amended and unamended condition for 3, 4 and 6 weeks.

		Incubation Period (weeks)					
		3		4		6	
		mgkg^{-1}					
Soil Type	Initial	Unamended	Amended	Unamended	Amended	Unamended	Amended
Alaba clay loam	8.8	-2.30**	56.63c*	-0.55	21.81c	-3.33	-3.34e
Kokate loam	3.5	-6.04	94.52b	-6.40	65.88b	-8.45	13.73c
Areka clay loam	2.5	-3.40	131.16a	-4.10	88.35a	-4.37	39.57a
Dilla silty soil	4.7	-1.76	134.57a	0.11	97.57a	-1.34	27.23b
Yirgalem clay loam	22	-0.72	61.12c	-274	21.81c	-2.55	8.60cd
Awassa clay loam	17	0.28	59.93c	-3.54	7.01cd	-1.08	2.93de
Ziway clay coam	28.5	4.39	23.27d	-1.91	-3.15	-1.92	-2.82
Debrezeitlay loam	23	2.24	22.23d	-1.98	0.63d	-2.10	-3.48
CV (%)	-	-	20	-	27	-	37

*****Means within column followed by different letters are statistically different from each other according to DMRT.

1.4. The effect of incubation period and $(NH_4)_2SO_4$ on soil pH change

As shown in Table 12, there was a decrease in the pH of all soils compared to the initial pH of each soil as nitrification proceeds with time. This is because the oxidation of one mole of ammonium produces four mole of H^+ acidifying the soil (Potash institute, 1979). Amending soils with $(NH_4)_2SO_4$ resulted in drastic decrease of pH of soils than same soils incubated without amendment. As pointed out above, ammonium is the source of H^+ and therefore the more the substrate the more H^+ ion produced resulting in reduced pH (Evas *et al.*,1998). Reduction in the pH of soil is one of the adverse effects of nitrification (Hayau and Kosuge. 1993).

Table 12. The effect of incubation period and $(NH_4)_2SO_4$ on the pH of different soils

Soil Type	Intial pH	With out $(NH_4)_2SO_4$			With $(NH_4)_2SO_4$		
		Incubation Period in weeks					
		3	4	6	3	4	6
Alaba clay loam	8.0	6.94	6.45	6.98	6.87	6.45	6.24
Kokate loam	6.2	5.22	5.1	5.0	5.78	4.9	4.82
Areka clay loam	6.2	5.55	5.1	5.08	5.7	4.87	4.77
Dilla silty soil	6.9	6.70	5.77	5.55	6.3	5.47	5.33
Yirgalem clay loam	7.5	7.26	6.62	6.39	6.89	6.0	5.79
Awassa clay loam	7.6	7.14	6.72	6.6	6.89	5.67	5.18
Ziway clay loam	9.1	8.66	8.37	8.35	8.2	8.15	8.1
Debrezeit clay loam	8.8	7.26	8.17	7.96	8.31	7.42	7.39
Mean	7.54	6.88	6.54	6.49	6.87	6.12	5.95
SE±	1.1	1.1	1.2	1.3	1.0	1.2	1.2

3.4. Relation between nitrification rate and soil properties

Table 13 presents correlation matrix among all soil properties. The first column shows the correlation between observed nitrate production in the laboratory and other

measured soil properties. It can be seen that nitrification rate was highly significantly ($P < 0.01$) correlated with initial soil pH ($r = 0.91$). The effect of initial soil pH as a major factor affecting nitrification rate of soils have been reported by several authors (Alexander, 1977; Focht and Verstraete 1977; Paul and Clark, 1989). Glimour (1984) found that that nitrification increase linearly with initial pH of soils over the range of 4.9 – 7.2. In a similar study, Hayatsu and Kosuge (1993) reported that the optimum initial soil pH for nitrification is between 7 and 9. In the present study the soils had intial pH value ranging from 6.2 to 9.1.

There are everal mechanisms which help nitrifying bacteria with stand or enable them to grow in alkaline codition (Edwards, 1990). According to this author sodium antiport is one of the mechanisms thet help nitrifiers maintain the internal pH low in alkaline environment.

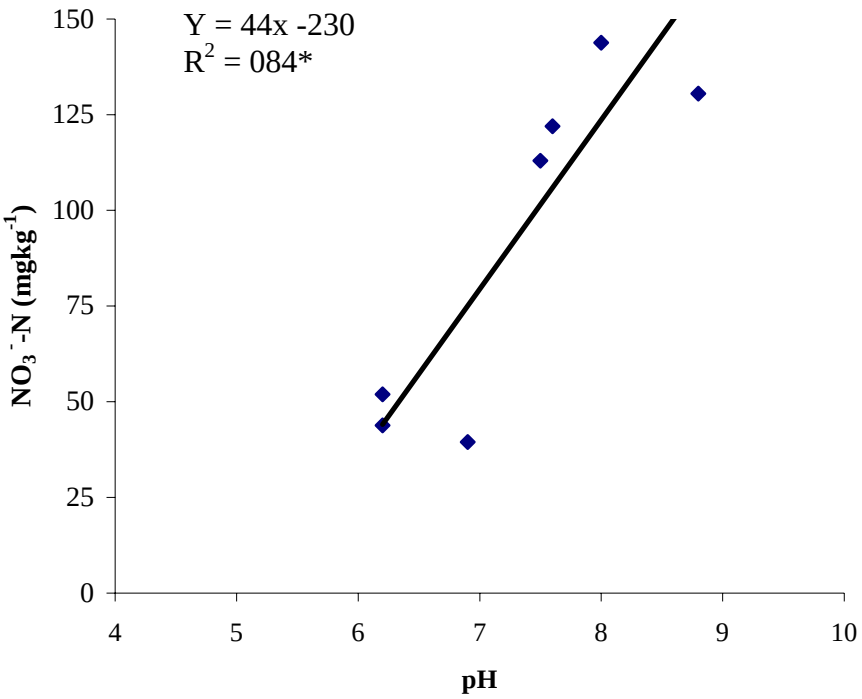
Table 13. Pearson correlation between soil Nitrate-N and soil properties and correlation values among all soil propeties.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	NO_3^-N	-																		
2	PH	0.91**	-																	
3	%TN	-0.49	0.20	-																
4	%OC	-0.55	-0.50	0.75*	-															
5	C/N	-0.68*	-0.72	-0.26	0.42	-														
6	Avp	0.47	0.63	-0.21	-0.49	-0.35	-													
7	Fe	0.23	0.33	0.60	-0.62	-0.01	0.84*	-												
8	Mn	0.30	0.05	-0.45	-0.21	0.42	-0.01	0.2	-											
9	Zn	0.18	-0.09	-0.33	0.19	0.28	0.11	0.4	0.74*	-										
10	Cu	0.48	0.71*	0.10	-0.37	-0.68*	0.82*	0.51	-0.05	-0.35	-									
11	Na	0.76*	0.78*	0.21	-0.32	-0.74*	0.34	-0.11	0.01	-0.28	.048	-								
12	K	0.79*	0.59	-0.17	-0.39	-0.29	0.22	0.04	0.52	0.19	0.12	0..58	-							
13	Ca	0.62	0.71*	0.51	0.03	-0.63	0.19	-0.30	-0.10	-0.40	0.41	0.91**	0.4	-						
14	Mg	0.46	0.76*	0.32	-0.11	-0.60	0.66	0.30	-0.46	-0.47	0..9**	0.53	0.08	0.62	-					
15	CEC	0.12	0.31	0.75*	0.42	-0.40	0.04	-0.40	-0.40	-0.51	0.31	0.55	-0.2	0.8**	0.58	-				
16	%BS	0.93**	0.96**	-0.05	-0.5	-0.63	0.51	0.21	0.21	-0.10	0.57	0.81**	0.76*	0.72*	0.62	0.23	-			
17	Clay	0.67	0.58	-0.39	0.54*	-0.23	0.46	0.35	0.35	0.07	0.35	0.41	0.88	0.18	0.22	-0.38	0.72*	-		
18	Sand	-0.13	0.17	0.09	-0.16	0.33	0.53	-0.49	-0.47	-0.33	0.52	-0.17	-0.05	0-0.25	0.26	-0.23	0.007	0.11	-	
19	Silt	-0.12	-0.32	0.08	0..32	0.32	-0.49	-0.47	-0.9	-0.46	-0.39	-0.02	-0.47	0.17	-0.1	0..5	0.28*	-0.6	0.8	-

* ** significant at 0.05 and 0.01 probability level respectivelly

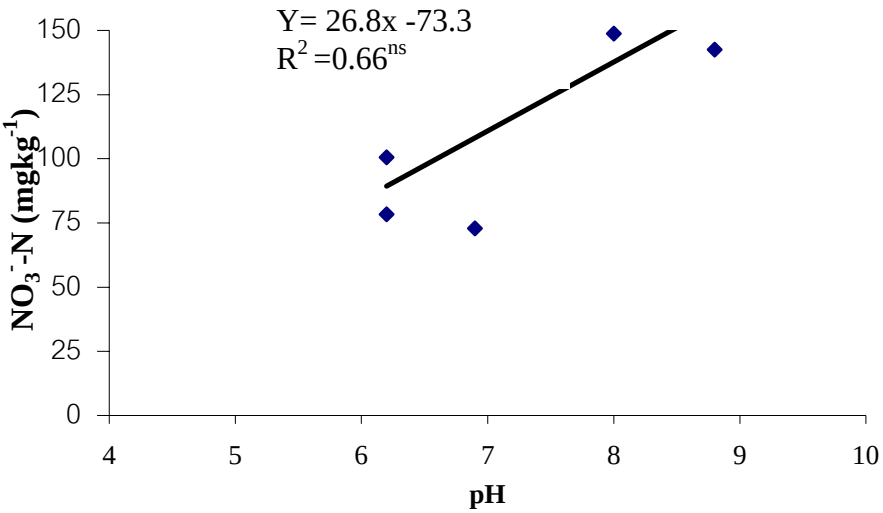
Nitrification was also found to be significantly and positively correlated with %BS (r = 0.93) and significantly Potasium (r = 0.79) correlated with and Sodium (r= 0.78). The positive correlation with % BS due to the fact that high base saturation indicates the availability of exchangeable cations which are essential nutrients for nitrifying bacteria (Wick *et al*., 1998).. Brady and Well (2002) stated that nitrification proceeds rapidly in soils where there is plenty of exchangeable cations. There was a significant and negative relation between nitrification rate and C/N ratio (r = -0.68). This is in agreement with the finding of Beuer *et al*., (2002) who reported that high nitrification activities were found for sites characterized by narrow C/N ratio and high carbon content in the mineral soils. Wide C/N ration promotes immobilization of nitrogen and thus the substrate for nitrification becomes limiting and hence reduce nitrification rate (Alexander, 1977).

Regression equation was calculated using initial soil pH and %BS as predictor and nitrification rate as dependent variable. The variables were selected based on their highly significant correlation coeficient with NO_3^--N produced. Fig. 1 a, b, and c depict the regression equation calculated using initial pH of test soils and nitrate-N produced at 3^{rd}, 4^{th} and 6^{th} weeks of incubation, respectively. Nitrification rate can significantly be predicted using initial pH of soils as explanatory variable with adjusted $R^2 = 0.84$. This significant prediction was obtained only when the nitrate-N produced at 3^{rd} week is used as dependent variable (Fig. 12a.). However, no significant correlation was found between initial pH of soils and nitrification rate when the NO_3^--N produced at the end of 4^{th} and 6^{th} weeks of incubation (Fig. 12b & c). There is a controversy about the predictive value of pH. For instance, Trolestra *et. al*., (1990) reported that pH can be used the predictor of net nitrification. On the contrary, Strong *et. al*., (1999) found that pH is not a good predictor of nitrification. In this study it was found that pH could be a good predictor of nitrification provided that a series of incubation is made up to 3 weeks or less up to 6 weeks or more. Otherwise it could be misleading to use he data from specified incubation period to derive regresion equation.

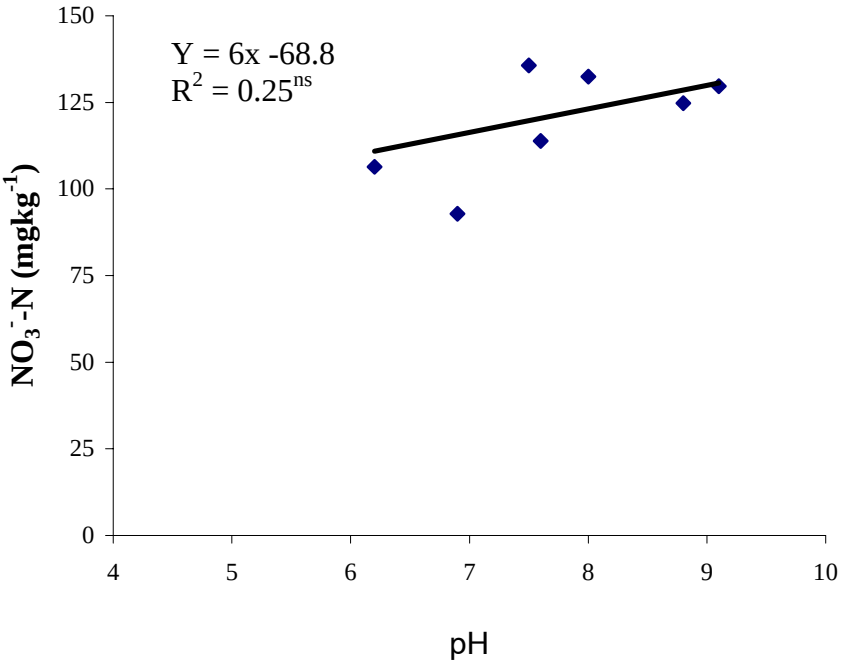


(a)

*Significant at 0.05 probability Level



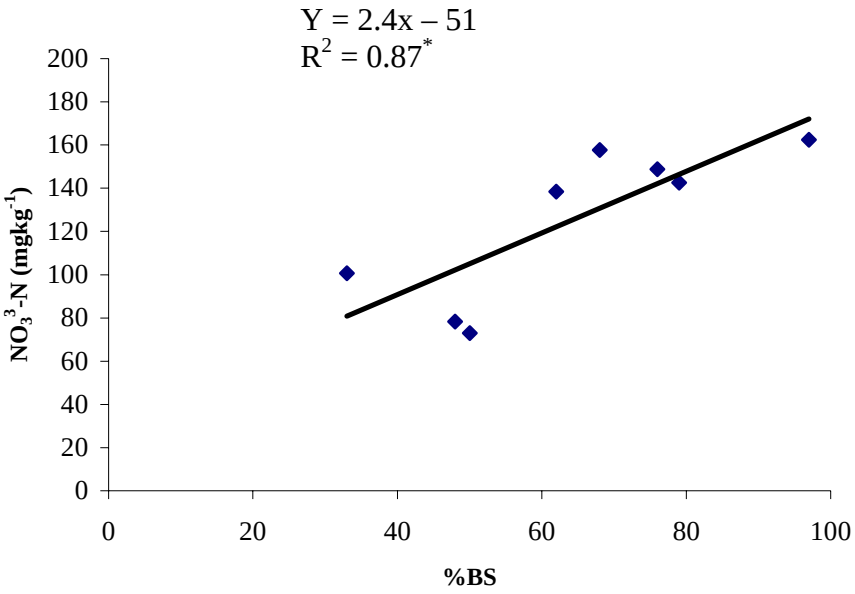
(b)



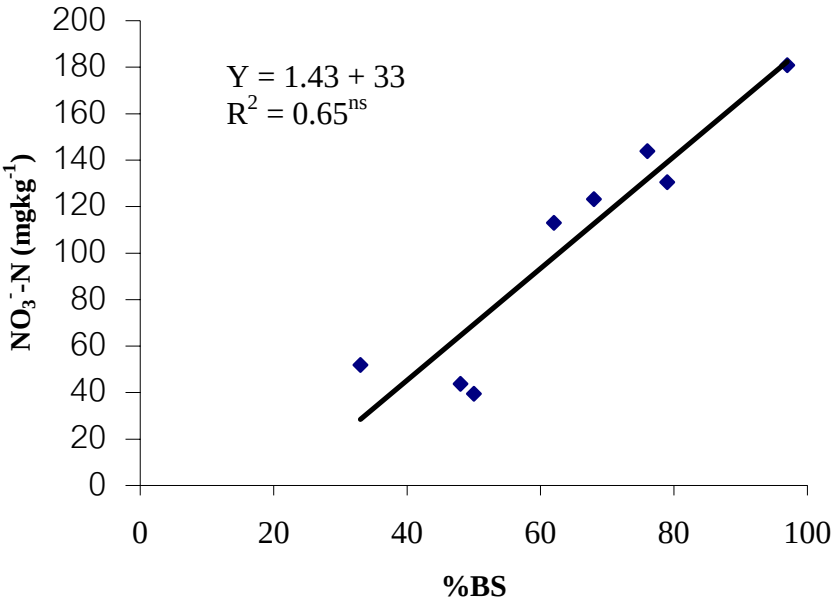
(C)

Figure 12. Linear relation between pH and NO_3^--N formed at 3rd week (a), 4th week (b)and (C) at 6th week.

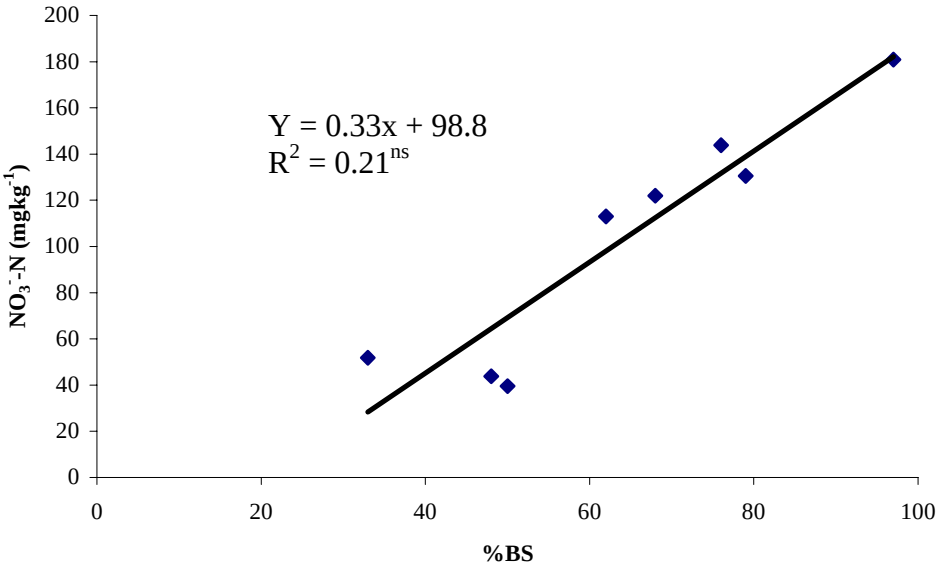
Similarly, nitrification rate in these soils could be predicted using %BS saturation as independent variable with adjusted R^2 = 0.87 (Fig. 13a). And this significant prediction was obtained when NO^-_3-N produced by the test soils at the end of 3rd week was used to calculate the regression equation. However, no significant relation between %BS and nitrification rate was found when NO^-_3-N produced at end of 4th and 6th weeks were used to calculate the regression equation (Fig. 13 b &c).



(a)



(b)



(c)

Figure 13. Linear relationship between %BS and NO_3-N produced at (a) 3rd week, (b) 4th week and (c) at 6th week

Experiment 2. Screening of Ethiopian traditional medicinal herbs for their nitrification inhibiting ability.

2.1. Preliminary Screening :Effect on pH, ammonium-N and nitrate-N content of the soil.

The effect of alcohol extracts of some Ethiopian medicinal herbs and commercial inhibitors on pH, NH_4^+-N and NO_3^--N contents of Awassa clay loam (Eutric fluvisol) soil after 2 and 3 weeks of incubation period is presented in Table 14. The tested herbs varied significantly in conserving N in the form of NH_4^+-N. At the end of 2nd week, 8 of herbs maintained significantly ($P< 0.01$) high amount of NH_4^+-N and low NO_3^--N in the soil which is an indication of nitrification inhibition ability (Mesinger *et al.,* 1980). The relatively low amount of NH_4-N in the soil treated with extracts of the remaining herbs and control is due to its oxidation to NO_3^--N. At the end of 3rd week, only 4 of herbs maintained significantly high amount of NH_4-N in the soil where their respective extracts were added as inhibitor. The herbs were *Eugenia caryophyllata, Echinops spp., Artemisa afra* and neem (*Azadiracta indica*). These herbs were previously reported to have antibacterial (killing bacteria) nature against human pathogenic bacteria (Mintesnot and Mogessie, 1999). Thus, their effectiveness as nitrification inhibitor in this study is probably due to their bactericidal property against ammonia oxidizing bacteria. *Eugenia caryophyllata* is well known for its germicidal property and it is a strong antiseptic and preservative. It is used to treat flatulence, colic, and indigestion and nausea. The occurrence of plant extracts and their exudates as nitrification inhibitor are also reported by several authors (Sahrawat and Mukerjee, 1977; Rice and Pacncholy, 1973; Paavolainen *et al*., 1998).

Eugenia caryophyllata performed as effective as neem but none of the herbs performed as effective as N-serve and DCD. Similar to the present finding, the significant nitrification inhibitory property of neem has been reported by several authors (Sahrawat and Parmar, 1975; Prasad and Power, 1995).

Table 14 The effect of alcohol extracts of some Ethiopian traditional medicinal herbs on the NH_4-N and NO_3^--N (mg kg^{-1})content of Awassa clay loam (Eutri fluvisol) soil amended with ammonium sulphate at the rate 50mg $100g^{-1}$ of soil and incubated at 25^OC.

	Incubation Periods (weeks)					
	2			3		
Medicinal Herbs / Inhibitors	pH	NH_4^+-N	NO_3^--N	pH	NH_4^+-N	NO_3^--N
Artemisa afra	6.65	89.7c*	43.5cd	6.50	42.2cd	56.3c
Cymbopogon citratus	6.60	68.1def	48.6cd	6.66	21.1g	73.2ab
Vernonia amygdalina	6.53	67.1def	56.0c	6.45	28.4efg	78.2ab
Croton macrostachyus	6.65	60.8ef	75.3a	6.43	21.2g	80.7a
Ruta chalaepensis	6.69	71.17de	49.4cd	6.90	40.0d	57.8c
Thymus serpyllum	6.50	55.4f	74.1a	6.50	24.3fg	79.9a
Echinops spp.	6.73	77.8cd	41.2e	6.80	51.4c	53.7c
Haginia abyssinica	6.52	75.5cd	52.6c	6.50	36.2de	73.2ab
Eugenia caryophyllata	7.14	127.6b	19.7f	7.0	92.9b	34.4de
Zingeber officinale	6.47	63.3ef	63.7b	6.50	34.5def	70.2ab
Neem (*Azadiracta indica*)	7.24	129.3b	22.1f	6.9	88.7b	38d
N-serve	7.5	137.1ab	16.8f	7.30	110.9a	29.8ed
DCD	7.3	145.5a	15.1f	7.23	114.2a	25e
Control	6.50	55.9f	69.5ab	6.43	26.4g	79.8a
CV (%)	-	8.5	9.05	-	12.17	8.99

*Means within columns followed by the different letters are significantly from each other at $P< 0.05$ according to DMRT

According to Prasad and Power (1995), epinibin, nibin, desacetylnimbin, salanin, desacetylacinin and azadiractin collectivelly know as melancins are chemical constituent of neem responsible for markedly retardation of nitrification.

In the study of these commercial inhibitors, N-serve and DCD were superior in their nitrification ability in inhibiting nitrification. These inhibitors retard nitrification by inactivation of cytochrome oxidase of ammonia oxidizing bacteria (Meisinger *et al*., (1980).

There was a decline in NH_4^+-N and a parallel build up of NO_3^--N in treated soil with all inhibitors with time, though the decline varies among herbs. This is due to the fact that inhibitors are organic compounds themselves and are subjected to the attack by microorganisms in the soil eventually leading to loss of inhibitory property (Yesuf and Vancleemput**,** 2000).

Effectiveness of Ethiopian medicinal herbs along with neem and commercial inhibitors expressed as percent inhibition of nitrification in Awassa clay loam soil is shown in Table 15. Of all herbs only *Artemisa afra*, *Echinops spp*., *Eugenia caryophyllata* and Neem have effectively inhibited nitrification. Averaged over two incubation periods, these herbs inhibited nitrification by 33, 37 and 64 and 60 % respectively. Comparatively, N-serve and DCD were superior and inhibited nitrification by 70 and 73% respectively.

The soil had initial pH of 7.6 and the pH of treated soil with different inhibitors were measured at the end of each incubation period. And the pH under each treatment is shown in Table 13 and those herbs and commercial inhibitors that were effective in inhibiting nitrification have maintained the pH at higher level than those of ineffective ones and the control. In all of the cases there was a decrease in pH compared to the initial soil pH. This is due to nitrification process resulting in the release of free H^+ ion (Tisdale *et al*., 1985).

Table 15 Nitrification inhibition percentage by Ethiopian traditional medicinal herbs.

Medicinal Herbs/Inhibitors	% Inhibition		
	Incubation periods (weeks)		
	2	3	Mean
Artemisia afra	37c*	29c	33
Cymbopogoncitratus	30dc	8d	19
Vernonia maygdalina	19e	0 d	10
Croton macrostachyus	0e	0d	0
Ruta chalaepensis	28cd	27c	28
Thymus serpyllum	0e	0d	0
Echinops spp	40c	32c	37
Haginia abyssinica	24d	8d	16
Eugenia Carophyllata	71ab	57ab	64
Zingeber officinale	8e	12d	10
Neem*(Azdiracta indica)*	68b	52b	60
N-serve	75ab	63ab	70
DCD	78a	68a	73
Control	-	-	-
CV(%)	13	23	-

*Means within columns followed by the different letters are significantly different from each other at P< 0.05 according to DMRT.

2.2. Secondary screening : Effect on pH, ammonium-N and nitrate-N content of the soil.

The effect of alcohol extracts of *Artemisia afra*, *Echinops* sp. and *Eugenia caryphyllata* and commercial inhibitors on NH_4-N and NO_3-N contents of soil is presented in Table 16. These 3 herbs conserved significantly ($P<0.01$) high amount of NH_4^+-N up to 4th week compared to the control. *Eugnia carophyllata* was the most effective inhibitor followed by *Echinops spp* and A*rtemisia afra* in that order. After the 4th week there was a decline in NH_4-N with a parallel increase in NO_3^--N with time in treated soils. This is due to the fact that the inhibitors themselves are organic compounds themselves and subjected to the attack by microorganisms eventually leading to loss of their inhibitory property (Yesuf and Vancleemput, 2000).

Effectiveness of nitrification inhibitors expressed as percentage inhibition over 3, 4 and 6 weeks of incubation periods is shown in Table 17. The highest degree of inhibition among herbs was achieved from *Eugenia caryophyllata*., followed by *Echinops sp*. and the least was *Artemisia afra*, at the end of 2nd week incubation period. This confirms the result obtained during the preliminary screening experiment in which *Eugenia caryophyllata, Echinops sp*. and *Artemisia afra* are effective nitrification inhibitors. Commercial inhibitors were superior in their percentage nitrification inhibition. For all inhibitors, the degree of nitrification inhibition was declined with time.

Table 16 The effect of Ethiopian medicinal herbs on the NH_4^+-N and NO_3^--N content in Awassa soil (Eutric fluvisol) amended with ammonium sulphate and incubated for various periods of time.

	Incubation periods (weeks)					
	2	3	4	2	3	4
Medicinal herbs/Inhibitors	NH_4^+-N (mg/kg)			NO_3-N (mg/kg)		
Artemis afra	78.81c*	39.12cd	34.7c	42.74b	56.09b	69.4b
Echinps spp	92.17c	52.31c	39.9c	41.9b	47.31b	54.4c
Eugenia caryophyllata	131.96b	102.44b	83.3b	22.39c	30.03c	38.5d
N-serve	150.89a	114.25ab	93.5ab	15.73c	25.7c	35.3d
DCD	156.19a	122.86a	96.3d	13.87d	23.87c	33d
Control	49.23d	39.11d	20.1d	70.24a	72.39a	81a
CV (%)	7.26	11.29	11.3	8.98	14.27	10.3

*Means within columns followed by the different letters are significantly different from each other at $P< 0.05$ according to DMRT.

Table 17 Nitrification inhibition percentage by Ethiopian traditional medicinal herbs.

Medicinal Herbs/Inhibitors	% Inhibition		
	Incubation periods (Weeks)		
	2	3	4
Artemisa afra	39c*	22b	14c
Echinops spp.	43c	35b	33b
Eugenia caryophyllata	68b	59a	38b
N-serve	78ab	64a	56a
DCD	80a	67a	59a
Control	-	-	-
CV (%)	9	17	20

*Means within column followed by the different letters are significantly different from each other at $P< 0.05$ according to DMRT.

2.3. Effect of nitrification inhibitors on soil pH.

The result of effect of herbal and commercial inhibitors on soil pH which was monitored in the secondary screening experiment is shown in Table 18. Both medicinal herbs and commercial inhibitors maintained the pH of the soil at the higher level than that of the control. This is probably due to the fact that the inhibitors prevented the oxidation of NH_4-N to NO_3-N as a result the production of H ions were suppressed. In a similar study, Yesuf and Vancleemput (2000) reported that inhibitor treated soils have higher pH than untreated ones. Thus, the nitrification inhibitors in addition to inhibiting nitrification, are also prevented the soil from acidification. However, the preventive capacity of inhibitors from acidification of soil decline with time. This is due to the fact that the

inhibitors loose the inhibitory ability due to degradation inhibitors themselves by microorganisms**.**

Table 18. The effect of nitrification inhibitors on the pH of Awassa clay loam (Eutric fluvisol) soil amended with 50 mg of ammonium sulphate and incubated at 25^{O}C.

Medicinal Herbs/ Inhibitors	Incubation periods (weeks)		
	2	3	4
Artemisa afra	6.9	6.8	6.7
Echinops spp	6.9	6.8	6.7
Eugnia caryophyllata	7.0	6.9	6.8
N-serve	7.4	7.3	6.9
DCD	7.3	7.3	7.0
Control	6.5	6.7	6.5
Mean	7.0	6.97	6.76
SE±	0.13	0.13	0.07

Experiment 3: <u>Nitrification inhibiting ability of Ethiopian medicinal herbs as affected by soil types</u>.

3.1. <u>Effect on ammonium-N and nitrate-N content of soil</u>

The effect of ethanol extracts of Ethiopian medicinal herbs including neem and commercial inhibitors on NH_4^+-N and NO_3^--N is presented in Table 19. All herbs maintained significantly high amount of NH_4^+-N in the soil with a parallel low amount of

NO_3^- -N at the end of second week compared to the control. Among herbs, *Eugenia caryophyllata*, neem, *Echinops* spp. *Haginia abyssinica Artemisia afra* and *Ruta chaelepensis* have maintained significantly high amount of NH_4^+-N in that order in soil where their respective extract were applied at the end of second week. The remaining herbs also maintained significantly high amount of NH_4^+-N compared to the control. Some of Ethiopian medicinal herbs were as effective as neem. None of the herbs superseded commercial inhibitors. The effectiveness of neem in conserving NH_4^+-N have been reported by Sahrawat and Parmar (1975) and Prasad and Power (1995). The amount of NH_4^+-N maintained in the treated soils declined at the end of 3rd week with a parallel build up of NO_3^- -N. However, the rate of decline varied among treatments and it was less for *Eugenial caryophyllata*, *Echinops* spp., neem, *Artemisia afra*, *Ruta chaelepensis* and commercial inhibitors. The decline in NH_4^+-N is due to the fact that the inhibitors decompose themselves resulting in loss of their ability to inhibit the oxidation of NH_4^+-N to NO_3^- -N (Yesuf and Vancleemput, 2002).

Table 19 The effect of Ethiopian medicinal herbs on the ammonium and nitrate nitrogen ($mgkg^{-1}$) content of Ziway and Awassa clay loam soils incubated for various period amended with 50 mg kg^{-1} of $(NH4)_2SO_4$.

Treatments	Incubation Period (weeks)			
	2		3	
	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N
Medicinal herbs/ Inhibitors				
Artemisia afra	114.6c*	62.5f	70.5e	104.8f
Cymbopogon citrates	74.2e	105.7bc	39.2g	134.2bc
Vernonia amygdalina	94d	89.8d	34.3g	128.1bc
Croton macrostachyus	69.4e	103.4c	37g	138.0b
Ruta chalaepensis	105c	64.2f	66.7ef	113.4e
Thymus serpyllum	90.8d	87.2d	60.5f	126.0cd
Echinops spp.	105.8c	76.7e	85.2d	94.5g
Haginia abyssinica	105.2c	69.3f	65.6ef	118.5de
Eugenia caryophyllata	151.9ab	43.2g	111.9bc	58.0ih
Zingeber officinale	70.5e	112.7b	39.9g	137.4b
Neem (*Azadiracta indica*)	145.3b	45.4g	106.8c	62.0h
N-serve	155a	42.6g	129.1a	50.5ij
DCD	158a	40.9g	118b	47.8j
Control	40.7f	127.4a	23.5h	154.7a
F-test (A)	*	*	*	*
Soil Types				
Ziway Clay Loam soil	124a	106.7a	89	154.5a
Awassa Clay Loam Soil	87.5b	46.3b	52.8	55.4b
F-test (B)	**	**	**	**
Inhibitors (A) X Soil Type (B)	**	**	**	**
CV (%)	7.4	9.4	8.3	7.0

*Means followed by the different letters are not statistically ($P<0.05$) different from each other according to DMRT. * ** Significant at 0.05 and 0.01 respectivelly.

3.2. Interaction effect of inhibitors and soil types on ammoniun-N and nitrate-N ontents of soil

Generally the the inorganic N (ammonium-N and nitrate-N) content of Ziway soil was much higher than that of Awassa soil irrespective of the treatments though both soils have been amended with equal amount of amonium-N in the form of ammonium sulphate solution. This difference could be attributed to the high amount of minerlizable organic matter content in Ziway than Awassa soil. However, the two soil types varied significantly in their NH_4^+-N and NO_3^--N content in response to the different inhibitor treatments across both incubation periods, the highest amount of NH_4^+-N was maintained in Ziway clay loam soil than Awassa soil due to inhibitors treatment. Inhibitor by soil type interactions were highly significant indicating that inhibitors behave differently in different soils with respect to conserving NH_4^+-N (Table 20).

Table 20 Interaction effect of Ethiopian medicinal herbs and soil type on NH_4^+-N and NO_3^--N content of Ziway and Awassa clay loam soils incubated for 2 and 3 Weeks at 25^0C and amended with 50 mg of $(NH_4)_2SO_4$.

Medicinal herbs/inhibitors	2 weeks				3 weeks			
	$NH4^+$-N		NO_3^-N		$NH4^+$-N		NO_3^--N	
	Ziway	**Awassa**	**Ziway**	**Awassa**	**Ziway**	**Awassa**	**Ziway**	**Awassa**
Artemisia afra	139.1bcd*	89.3f	81.4f	43.5lm	98.9e	41.75ghi	153.3ed	56.0k
Cymbopogon citrate	80.4 fg	68ghi	162.9b	48.57klm	57.27f	21l	195.2b	73.3ij
Vernonia amigdalina	120.7e	67ghi	123.6c	56.0jk	40.3ghij	28.4jkl	179.7c	77.3ihj
Croton macrostachyus	78.6fg	60.2ij	131.4c	75.4fghi	52.9fg	21.2kl	195.4b	83.0hi
Ruta chalepensis	138.8cd	71.7ghi	78.9fgh	49.4klm	93.4e	39.9hij	169.0cd	56.4k
Thymus serpyllum	126.4de	55.4j	100.3e	74.1ghi	96.6e	24.3kl	172.1b	80.6hij
Echinops spp.	134.4cde	77fgh	112.3d	41.1m	119.1bcd	51.4fgh	141.9b	47.0k
Haginia abyssinica	134.9cde	75.5fgh	85.f	52.8kl	95e	36.2ijk	163.8de	73.8ij
Eugenia caryophyllata	175.4a	127.6de	66.6ij	19.7N	130.9b	92.8e	91.5gh	26.8l
Zingeber officinale	77.6fgh	63.3hij	161.6b	63.7ij	45.3fghi	34.5ijk	204.6b	74.6ij
Neem	161.4b	71.7ghi	68.6hi	22.1N	125.3bc	88.4e	99.1g	25.9l
N-serve	172.6ab	129.3de	68.5hi	16.8N	147.3a	110.9d	84.5hi	17.26l
DCD	170ab	147.4c	66.8ij	15.1o	123.5bc	114.4d	79.9hij	16.3l
Control	25.51k	55.9j	185.5a	69.48hi	20.6l	26.4kl	229.6a	78.3lhj

*Means followed by the same letters are not statistically different from each other according to DMRT.

Effectiveness of Ethiopian tradition medicinal herbs expresed as percentage of nitrification inhibition is shown on both Ziway and Awassa clay loam soil in Table 21. At the end of 2nd week the Percenatge of nitrificating inhibion varied between 10 and 67 % for medicinal herbs. *Eugenia caryophylata* ranked first in percenatge of nitrificating inhibition followed by neem, *Artemisia afra*, *Ruta chaelepensis* and *Echinops* spp. There was drastic decline in percentage of nitrificating inhibition for most of the herbs at the end of 3rd week.

At the end of both incubation periods the highest percenatge of nitrification inhibition was achieved in Ziway clay loam soils than Awassaa soil. In a similar study McCarty and Bremner (1989) compared the effectiveness of inhibitors in 3 soils of USA. They found that 6 of the 11 compouds to be effective ingeneral and the highest percentage nitrification inhibition was achieved uin Stroden soil followed by Webster soil and the least in Harden soil. This implies that relatively, the application of nitrification inhibitors could be more beneficial in Ziway soil than Awassa soil. The differences in the effectiveness of inhibitors in these soils is probably due to their differences in physical and chemical characterstics suggesting that Ziway soil have soil chracteristcs that favor the effectiveness of inhibitors. Soil organic matter, intial soil pH, temperature and the nitrifying ability of soils are some of the factors affecting effeciveness of nitrification inhibitors (Keeney, 1980).

Table 21 Effectiveness of Ethiopian traditional medicinal herbs in two soils expressed as Percentage of nitrification inhibition after 2 and 3 weeks of incubation.

Treatments	Incubation Priod (weeks	
	2 weeks	3 weeks
	Percentage inhibition (%)	
Medicinal herbs/Inhibitors		
Artemisia afra	46.5b	30.7de
Cymbopogon citrates	21.0d	12.2gh
Vernonia amygdalina	26.2d	10.8gh
Croton macrostachyus	14.3e	7.7h
Ruta chalaepensis	42.8bc	25.5e
Thymus serpyllum	22.8d	14.3fg
Echinops spp.	40.2bc	35.1d
Haginia abyssinica	39.0c	18.0f
Eugenia caryophyllata	67.7a	58.9bc
Zingeber officinale	10.3a	11.0gh
Neem (*Azadiracta indica*)	65.3a	55.0c
N-serve	69.5a	62.9ab
DCD	71.0a	66a
Control	-	-
F-test (A)	*	*
Soil Types		
Ziway Clay Loam soil	45.5	35a
Awassa Clay Loam Soil	37b	27b
F-test (B)	*	*
Inhibitors (A) X Soil Type (B)	**	**
CV (%)	12.0	12.9

Means followed by the same letters are not statistically different from each other according to DMRT. * ** Significant at 0.05 and 0.01 respectivelly

Interaction effect of Ethiopian medicinal herbs including neem (none Ethiopian medicinal herb) and commercial inhibitors by soil type on the percentage of nitrification inhibition is shown in Table 22. The inhibitors achieved different percentage nitrificating inhibition in the two soils. For example *Artemisa afra* inhibited nitrification by 56 % in

Ziway soil but by 37 % in Awassa soil at the end of 2nd week. Similarly, *Thymus serpyllum* have inhibited nitrification by 45% in Ziway soil and literaly none in Awassa soil. This may due to the fact that the ammonium oxidizing bacteria that are present in Ziway soil could be more sensitive to inhibitors than those that occur in Ziway soil (Keeney, 1980).

Table 22. Intraction effect of Ethiopian medicinal herbs and soil types on the on the Percentage of nitrifating inhibition.

Medicinal herbs/Inhibitors	Incubation Period (weeks)			
	2		3	
	Ziway	Awassa	Ziway	Awassa
Artemisia afra	56de*	37ghi	33ef	28fg
Cymbopogo ciratus	12lm	30ij	15ih	9i
Vernonia amigdalina	33hij	19kl	33ef	19gh
Croton mcrostachyus	30ij	9mn	15ih	10i
Ruta chaelepensis	57de	28ij	24gh	26fg
Thymus serpyllum	45fg	0n	28fg	0j
Echinops spp.	39gh	39gh	38fg	31ef
Haginia abyssinica	53ef	24jk	28fg	8i
Eugenia caryophyllata	63cd	71abc	60bcd	57cd
Zingeber officinale	12lm	8mn	12i	10i
Neem	62cde	68bc	57cd	53d
N-serve	63cd	75ab	63abc	62abc
DCD	64cd	78a	65ab	68a

*Means followed by the same letters are not statistically different from each othe according to DMRT.

3.3. The effect of Ethiopian traditional medicinal herbs on pH

The Ziway and Awassa soils had initial soil pH of 9.1 and 7.6 respectively. The pH of both soils after treated with ethanol extracts of herbs and commercial inhibitors and incubated for 2 and 3 weeks is shown in Table 23. The reduction in pH due to nitrification was lower for treated soils than the control. For instance at the end of 2nd week, it was 8.7 for most treated soils of Ziway while more than 6.6 for treated soils of Awassa. Where as The pH has dropped from 9.1 to 8.4 and 7.6 to 6.4 in control soils of Ziway and Awassa respectively. This is due to the fact that the inhibitors have inhibited the oxidation of NH_4^+N to NO_3^--N. In a semilar study, Yesuf and Vancleemput (2001) reported that nitrification inhibitor treated soils have higher pH than untreated soils.

Table 23 The effect of Ethiopian traditional medicinal herbs on pH of two soil after 2 and 3 weeks of incubation period.

Medicinal herbs/Inhibitors	Ph			
	Ziway clay loam Soil		Awassa clay loam soil	
	2	3	2	3
Artemisia afra	8.7	8.5	6.7	6.5
Cymbopogo ciratus	8.6	8.1	6.6	6.4
Vernonia amigdalina	8.7	8.5	6.6	6.6
Croton mcrostachyus	8.7	8.3	6.7	6.4
Ruta chaelepensis	8.7	8.2	6.5	6.9
Thymus serpyllum	8.6	8.5	6.7	6.5
Echinops spp.	8.7	8.5	6.7	6.5
Haginia abyssinica	8.7	8.7	6.5	6.5
Eugenia caryophyllata	8.8	8.6	7.1	6.9
Zingeber officinale	8.4	7.8	6.5	6.5
Neem	8.8	8.5	7.0	6.7
N-serve	8.8	8.7	7.4	6.9
DCD	8.9	8.9	7.2	7.0
Control	8.5	8.4	6.5	6.4
Mean	8.68	8.44	6.76	6.62
SE ±	0.020	0.035	0.081	0.1

Experiment 4: The effect of nitrification inhibitors on yield and N-use efficiency of wheat

4.1 Yield and attributes

The effects of both herbs based and commercial inhibitors and nitrogen fertilizer on the yield and yield components are shown in Table 24. The inhibitors and application of increasing level of N have significantly ($P<0.001$) increased the grain and straw yield, plant height, number of tillers and spike length of wheat. *Eugenia caryophyllata*, Neem, N-serve and DCD increased grain yield by 7, 7, 10 and 13% over the control respectively. And the straw yields by 14.8, 12, 16.6% and 26.8% over the control respectively. There are several reports indicating that nitrification inhibitors increase improve the yield and yield components of many crops (Meseinger *et al*, 1980; Prasad and Power, 1995; Prasad and Power, 1997; Prasad and Power, 1997). Bock (1987) obtained a 19 to 47% increase in the yield of wheat with the application of basal NO_3^--N + urea + N-seve compared to NO_3^--N alone. A 15- 18% increase in the yield of sorghum was reported by Camberato and Bock 1989). According to International Rice Commission (,) ammonium sulphate treated with N-serve increased the yield of rice by 15-20% over untreated control. The inhibitors treated plants had significantly higher plant height, tiller numbers and spike length compared to the control. Inhibitor treated wheat plants had higher number of tillers and that is one of the mechanism by which nitrification inhibitors improve the yield of crops. In an other study Kumar *et. al*., (2000) found that 10% DCD + 95% urea produced taller plants and significantly more number of primary branches in Pusa variety of mustard compared to urea alone.

Applications of increased level of N have significantly and dramatically increase the yield and yield components of wheat. Nitrogen levels of 23 46, 92 138 $kgha^{-1}$ increased the grain yield by 44.8, 83, 198 and 428% respectively. And straw yield 68, 135, 264 and 375% respectively.

Table 24 The effect of nitrification inhibitors and N fertilizer on the yield and Yield components of wheat

Treatment	Grain Yield ($gpot^{-1}$)	Straw Yield (gpo^{t-1})	Plant height (cm)	Number of Tillers	Spike length (cm)
Inhibitors					
I0	6.90c	10.8c	63.b	18c	5.9b
I1	7.38b	12.4b	64.9ab	23ab	7.0a
I2	7.38b	12.1b	63b	22ab	5.9b
I3	7.63ab	12.6b	66.4a	21c	6.0b
I4	8.00a	13.7a	64.7ab	25a	6.6b
F-test	*	*	*	*	*
N-fertilizer					
N0	2.97e	4.58e	50.6e	0.0e	3.7e
N1	4.30d	7.7d	61.7d	11d	4.9d
N2	5.44c	10.8c	66.2c	22c	6.1c
N3	8.86b	16.7b	74.7a	27b	7.5b
N4	15.71a	21.8a	69b	48a	9.6
F-test	*	*	*		*
I X **N**	ns	ns	ns	ns	ns
CV (%)	8.6	8	5	20	10

Nitrogen is the most important yield limiting factor in Ethiopia and its application is taken as a key measure to increase crop production. However, blanket applications of 100 $kgha^{-1}$ DAP and 50 $kgha^{-1}$ urea for all cereals and soils practiced for many years. Recently, attempt is being made to develop site-specific fertilizer recommendation. In

this line Taye *et al*., (2000) recommended 92 kg Nha^{-1} for wheat growing in Arisi province of Ethiopia. In this study the yield increased up to 138 kg ha^{-1} linearly indicating that still applying N beyond that recommended rate for Arsi area by the above authors does not satisfy the Awassa soil for wheat production. This implies that consolidated effort should be made to develop site specific fertilizer recommendation by soil type and crops. Inhibitors by N levels interactions were not significant suggesting that the effect of inhibitors is not affected by change in the levels of N applied or vice-versa. This is in agreement with Hergert and Wiese (1984) who reported that the interaction between N-serve and N rate were non significant.

4.2 The effect of nitrification inhibitors and N levels on N-uptakes

Table 25 shows the effect of nitrification inhibitors and different levels of N fertilizer on %N and total N-uptake per pot of wheat. Nitrification inhibitors and increasing levels of N fertilizers have significantly increased GN%, SN%, Grain N, GN and SN uptake. *Eugenia caryophyllata*, neem, N-serve and DCD improved GN% by 10, 9, 11, and 17% respectively. The correspondingly, SN% of was increased by 11, 11.7, 15 and 12 %.

Table 25 The effect of nitrification inhibitors on the nitrogen Parameters

Treatment	Grain N	Straw N	Grain N	Straw
	%	%	$mgpot^{-1}$	$mpot^{-1}$
Inhibitors				
I0	1.44c	0.26b	106b	32.8b
I1	1.54b	0.29a	116ab	41.5a
I2	1.53b	0.29a	116ab	43.2a
I3	1.55ab	0.30a	118a	40.5a
I4	1.61a	0.29a	126a	44.6a
F-test	*	*	*	*
N-fertilizer				
N0	1.31d	0.14d	37.8e	64e
N1	1.45c	0.27c	61d	21d
N2	1.57a	0.32b	84c	35c
N3	1.67a	0.36a	144b	61b
n4	1.71a	0.37a	258a	81a
F-test	*	*	*	*
I X **N**	ns	ns	ns	ns
CV (%)	6	11	11	13

A 10 to 30% increase in GN% was obtained by successively increased level of N. SN% was increased with between 92 and 164%.

4.3. The effect of nitrification inhibitors and N levels on wheat N content at booting stage.

The effect of nitrification inhibitors on percent N content of wheat at booting stage is shown in Figure 14. The inhibitors significantly ($P < 0.001$) affected the N content of wheat at booting stage. Both herbs based and commercial inhibitors significantly increased the N content of wheat compared to the control. Dicyandiamide was found to be superior in increasing the percent N content compared to the other treatments. N-serve, *Eugenia caryophyllata* and Neem (*Azadiracta indica*) increased the N content of at booting stage in that order. The increment N content of expressed as percentage is due to maintenance of applied N in the form of ammonium N (which is less labile to loss) for long during the growing period.

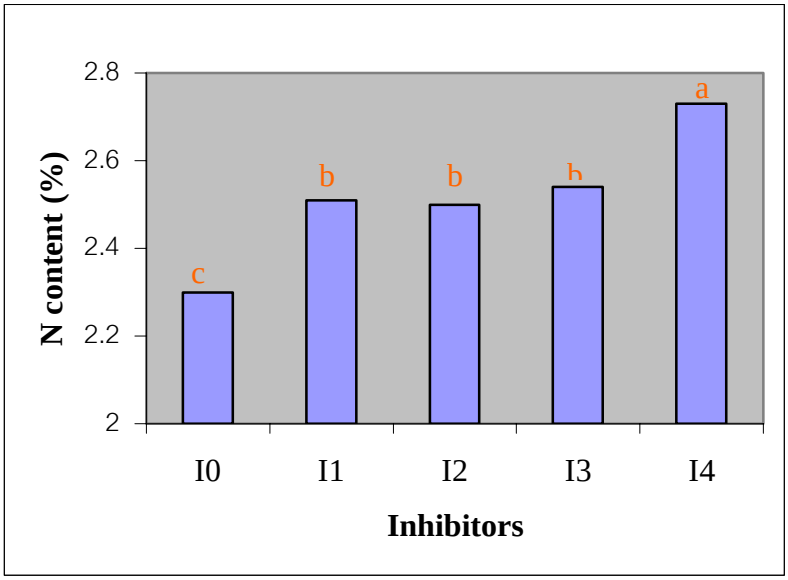


Figure 14. The effect of nitrification inhibitors at booting stage

In a similar study, Freney *et al*., (1992) reported that inhibitor treated wheat accumulated significantly high nitrogen in the plant tops. According to these authors nitrification inhibitors prevented the oxidation of ammonium-N, prevented nitrogen loss by denitrification and increased the accumulation of N by wheat plants.

The effect of increasing levels of N on percent N content of wheat at booting stage is shown in Figure15. Increasing levels of N-fertilizer increased the percent N content of wheat significantly on plant tops at booting stage. The highest amount of N content was obtained from plots that received 138 $kgha^{-1}$. of N And the least from 23 $kgha^{-1}$ of N.

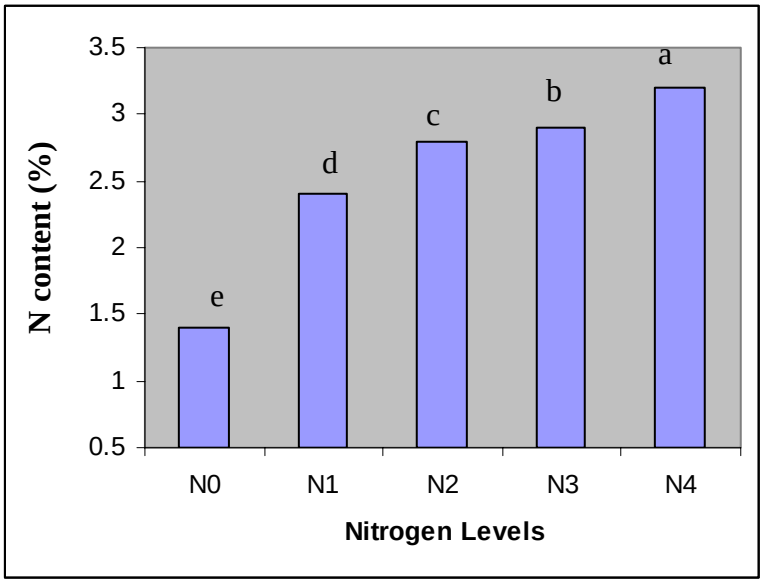


Figure 15. The effect of N levels on N content of wheat at booting stage**.**

The linear increase in percent N content of wheat suggests that the optimum amount N required for growing wheat in Awassa soil is not achieved at the highest level of applied

N that is 138 $kgha^{-1}$ in this experiment. Inhibitors by N levels interaction was not significant suggesting that the effect of inhibitors on N content is not affected by the change in the levels of N or vice-versa.

4.4. The effect of nitrification inhibitors and N fertilizers on N recovery efficiency of wheat.

The effect of nitrification inhibitors on N recovery by wheat is shown in Figure 16. Both herbal and commercial inhibitors have significantly increased the N-recovery by wheat. The N-recovery by wheat in none inhibitor treated soil was 60% and this was increased to 79% by *Eugenia caryophyllata*, to 78% by neem, to 82% by N-serve and 90% by Dicyandiamide (DCD). In a similar study, Freney *et. al*. (1993) reported that addition of nitrification inhibitor phenyacetylene increased the N-recovery from 57% in untreated control to 74%by N-serve, to 78% by coated calcium carbide and to 92% by ethynylpyridine. In an othe study, Freney *et al.*, (1992) found that wax coated calcium carbide applied to wheat together with urea resulted in 46% greater recovery of applied nitrogen in plant and soil system at harvest.

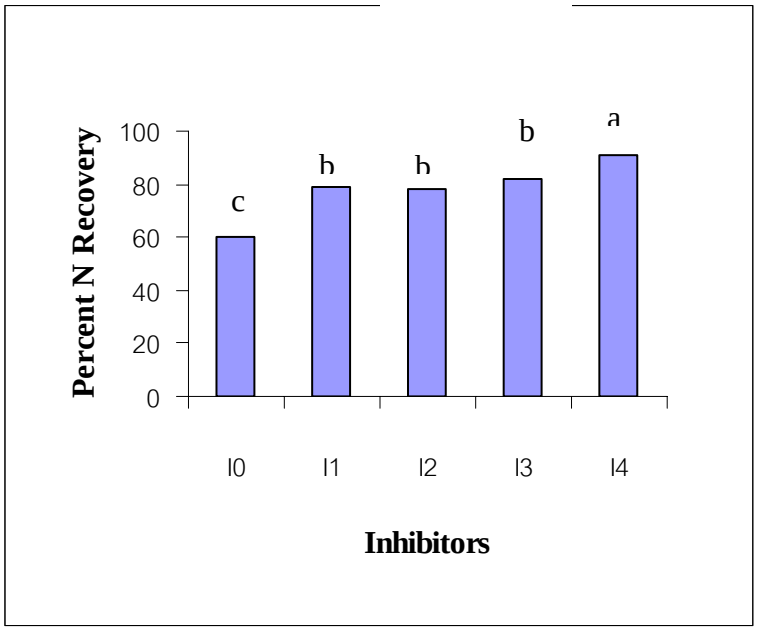


Figure 16. The effect of nitrification inhibitors o N recovery of wheat.

The effect of N levels the N-recovery by wheat is shown in Figure17.Each successive increase in the applied N increased N-recovery significantly and linearly. The N-recoveries for the applications 23, 46, 92, 138 N kgha^{-1} were 70, 75, 80 and 92% respectively. The N-recoveries obtained in this study are generally higher than that can be obtained in the field studies. This is due to the fact that the experiment was conducted in the greenhouse where most other growth factors were maintained at ambient condition than that could occur in the field or natural condition This assumption is substantiated by the report of Allison (1965) who stated that in pot culture experiments the recoveries of applied N have generally been high and ranges between 30% and 92%. The other possible reason for high recovery efficiency is probably that the N rate is calculated for this pot experiment is based on that the rate from per hectare basis and it is small for pots.

Inhibitors by N level interactions were none significant.

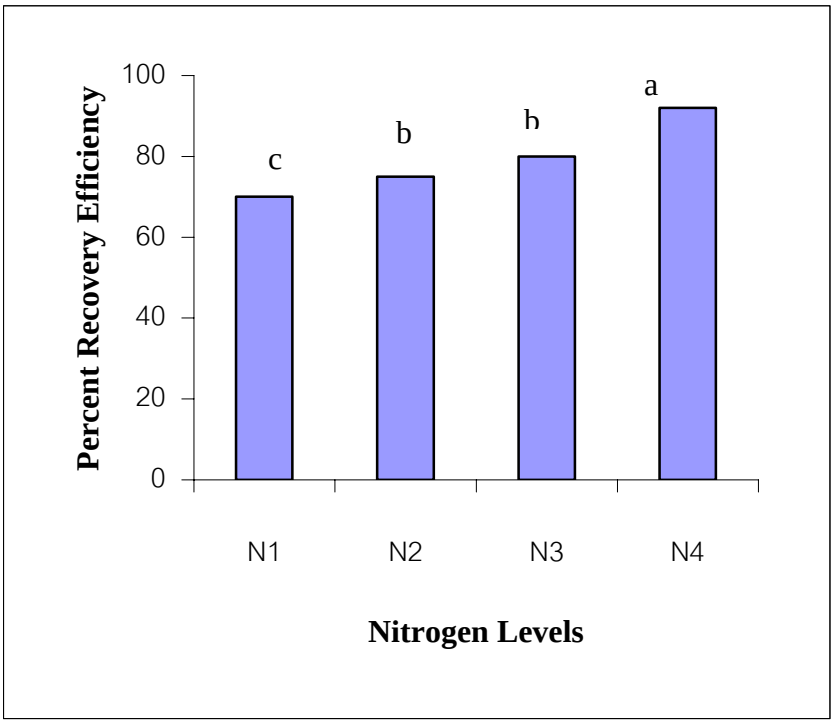


Figure 17. The effect of N fertilizers on N recovery of wheat.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of laboratory and greenhouse experiments the following conclusions could be drawn:

Investigation on the nitrification potential of some soils occurring in southern and central Ethiopia.

Under this experiment the nitrifying ability of eight mineral soils occurring in the southern and central Ethiopia was investigated and it was founds that the soils vary significantly and widely in their nitrification potential. Accodingly Ziway clay loam (Fluvi-vitric Andisol), Alaba clay loam (Plani-vertic Cambisol), Yirgalem clay loam (Eutric Nitisol), Debrezeit loam (Vitric Andisols) and Awassa clay loam (Eutric Fluvisol) soils produced the highest amount of nitrate-N at the end of 4 weeks of incubation under both ammonium sulphate amended and unamended condition. And these soils are categorized as fast nitrifying soills in comparision with Kokate loam (Dystric Nitisols), Areka clay loam (Haplic Alisol) and Dilla silty (Rhodic feralsol) soils. These soils are categorized as slow nitrifying soils. This implies that these soils maintain nitrogen in the form of ammonium-N for long period of time, which is less subject to loss. Which ultimately suggests that crops growing in these soils can use the nitrogen coming from soil organic sources or from fertilizer effectively.

The variation in the nitrifying ability of the test soils is attributed to their difference in pH, %BS, Na, K and C/N ratio. PH and %BS could be used to predict the nitrifying ability of these soils provided the data of nitrate-N is obtained from a series of incubations. It is also recommended that the real picture of nitrifying ability of soils is obtained provided that a series of incubations starting from for three weeks up to for six weeks or more are made.

Screening of Ethiopian traditional Medicinal herbs for their nitrification inhibiting ability.

Out of Ethiopian traditional medicinal herbs tested in this experiment, three of them namely *Eugenia caryophyllata*, *Echinops spp*. and *Artemisa afra* were identified to be nitrification inhibitors. They were found to be effective up to the end of fou weeks of incubation. The highest percentage of nitrification inhibition was achieved at the end of two week and the least at the end of 4th week. *Eugenia caryophyllata* was ranked the best among Ethiopian traditional medicinal herbs and gave comparable result as that of Neem in inhibiting nitrification. However, none of the herbs outshined the commercial inhibitors. Application of both herbal and commercial inhibitors together with ammonium sulphate prevented the soil from acidification compared to the soil that received ammonium sulphate alone. Thus, alcohol extracts of these herbs could be blended with ammonium containing fertilizers to increase N-use efficiency of crops.

Nitrification inhibiting ability of Ethiopian medicinal herbs as affected by soil types

Over two soil types, the Et hiopian medicinal herbs *Eugenia caryophyllata*, *Artemisia afra*, *Ruta chaelepensis* and *Echinops* spp. were identified as the most promising nitrification inhibitors. *Eugenena caryophyllata* was raked as the best among Ethiopian medicinal herbs and gave comparable result as that of Neem. Comparing soil types, The highest percentage inhibition was achieved in Ziway clay loam (45%) than Awassa clay loam (37%) at the end of two weeks of incubation period. The corresponding percentage nitrification inhibition at the end of three weeks of incubation were 37 and 27%. This implies that application nitrification inhibitors of any type with ammonium fertilizers could best be used in Ziway than Awasssa soil.

Inhibitors by soil type interactions were significant suggesting that there is a need to identify and recommend specific inhibitor for a specific soil.

The effect of nitrification inhibitors on yield and N-use efficiency of wheat.

From the results this experiment it can be concluded that alcohol extracts of both *Eugenia caryophyllata* and neem and commercial inhibitors significantly improved the grain and straw yield and plant height, number of tillers and spike length of wheat. *Eugenia caryophyllata*, neem, N-serve and DCD increased the grain yield by 7, 7, 10 and 13% respectively. And the straw yield by 14, 8, 12, 16 and 28% respectively. The N-recovery efficiency was increased from 60% in the control to 78% by *Eugenia caryophyllata*, to 79% by neem, to 82% by N-serve and to 90% by DCD. Though the herbs performed less effective to the commercial inhibitors, they significantly improved the all parameters of wheat. Thus, as these herbs are available and cheap, they could be blended with ammonium fertilizers to improve N-use efficiency of crops. They are particularly applicable to subsistent farmers who cannot afford to buy commercial inhibitors. However, further extensive laboratory and field studies are required. On the other hand, to the authors' knowledge there has never been experience of testing and using commercial inhibitors in Ethiopia. Thus, this study proved that commercial inhibitors that are N-serve and DCD were found to be effective inhibitors. These inhibitors could be imported and used by large scale private farmers who can afford the expenses.

Increasing levels of N increased the yield, yield components and N-recovery of wheat significantly. And the highest amounts of all parameters were obtained from the application of the highest level of N which is 138kgha^{-1}. This implies that there is a need to go further than this rate to attain the biological optimum for wheat production in Awassa area.

LITERATURE CITED

AHI (African Highland Initiative). 1998. **Annual report**. Uganda.

Alexander, M. 1977. **Soil microbiology**. 2nd edition, John Wiley & and Sons , New York. 467p.

Alexander, M. 1965. Nitrification. Pp. 307-343. In: W. V. Bartholomew and F. E. Clark (eds.) **Soil Nitrogen**. ASA, Madison.

Amere, G. 1976. **Some common medicinal and poisonous plants used in Ethiopian folk**, 47p.

Amsal, T. and G. Tanner . 2001. Effects of fertilizer application on N and P uptake, recovery and use efficiency of bread wheat on two soil types in central Ethiopia. **Ethiopian J. of Nat. Res.** 3: 219-244.

Armstrong, A.C. and T.P, Burt .1993. **Nitrate losses from agricultural land and nitrate process, pattern and management**. John Wiley and Sons, New York.

Azam, F. and S. Farooq. 2003. Nitrification inhibition in soil and ecosystem functioning- An Overview. **Pakistan J. Biol. Sci**. 6:528-535.

Azene, B., A. Birnie and B. Tengnas. 1993. **Useful trees and shrubs for Ethiopia: Identification, propagation and management for agricultural and pastoral communities**. Regional Soil conservation unit/ SIDA 474p.

Belser, L. W. 1979. Population ecology of nitrifying bacteria. **Ann. Rev. Microbiol**. 33: 309-333.

Brady, N. C. and R. R. Well. 2002. **Nature and Properties of Soil**. Prentice Hall, New Jersey. 960p.

Breitenbeck, G. A., A. M. Blackmer and J. M. Bremner. 1980. Effect of different nitrogen fertilizers on emmision of nitrous oxide from soil. **Geophysic. Res**. 7: 85-88.

Breuer, L, R. Kiese and K. Butterbach-Bahl. 2002. Temperature and moisture effects on nitrification rates in tropical rain-forest soils**. Soil Sci. Soc. Am. J**. 66: 834- 844.

Briggs, G. G. 1975. The behavior of nitrification inhibitor "N-serve" in broadcast and incorporated application to **Soil. J. Sci. Food Agric**. 26: 1083-1092.

Brock, B. R. 1987. Increase in maximal yield of spring wheat by maintaining relatively high ammonium/nitrate ratio in soil. **J. Fertil. Issue**. 4: 68-72.

Brock, T. D. and T.M. T. Madigan. 1994. Biology of Microorganisms. 5th edition, Prentice Hall, New Jersy.

Bundy, L. .. G and J. M Bremner. 1973. Inhibition of nitrification in soil**. Soil Sci. Soc. Proc**. 37: 396-398.

Camberato, J. J and B. R. Bock. 1989. Response of grain sorghum to enhanced ammonium supply. **Plant and soil**. 113: 78-83.

Campbell, C. A., R. J. K. Myers and D Curtin. 1995. Managing nitrogen for sustainable crop production. **Fert. Rs.** 42: 277-296.

Coulbe, E.C, Wilding, L.P and Dixon, J.B. 1996. Overview of vertisols: Characteristics and impact on society. **Ad. Agro**n. 57: 289-375

Coyne, M. 1999. **Soil microbiology: An exploratory approach**. Delmar publisher, Washington, 462p.

De Boer, W., P. J. A. Klien Gunnewiek and S. R. Troelsera.1990. Nitrfication in Dutch heathland soils II. Characteristics of nitrate production. **Plant Soil** 127: 193-200.

De Boer, W. P. J. A, Klien gunnewiek ,S.R , Troelstera and H. J. Laanbroek .1989. Two Types of Chemolithotrophic Nitrification in acid Heathland humus. **Plant Soil**. 119: 229-235.

Devakumar, C.1986. **Identification of nitrification retarding principles in Neem (*Azadiracta indica* Juss) seeds**, PhD thesis, Indian Agricultural Research Insitute, New Delhi

Degrange, V., M. M. Couteaux, J. M. Anderson, M. P. Berg and R. Lensi. 1998. Nitrification and occurrence of nitrobactor by MPNP- PCR in low and high nitrifying coniferous forest soils**. Plant and soil** 198: 201-208.

Edwards, C. 1990. **Microbiology of Extreme Environments**. Open University press, Great Britain. 218p.

Englestand, O.P. 1985. **Fertilizer technology and use** . 3rd edition, SSSA, Madison.

Esatvillo, J. M, M. Rodriguiz and C. Gnzalez-Murua. 1996. Nirtogen losses by denitrification and leaching in grass land . Fer. Res. 43: 197-201.

Ethiopia Agricultural Research Organization (EARO). 2001. **Soil and water research program strategy.** Addis Ababa.

Evans, C. M., M. K. Conyers, A. S. Black and G. J. Poile. 1998. Effect of ammonium, organic amendments and plant growth on soil pH stratification. **Aust. J. of Soil Res**. 36: 641-653.

FAO. 2001. Statistical database. http://www.fao.org.

Flessa, H., W. Pfau, P. Dorsch and F. Bees. 1996. The effect of nitrate and ammonia fertilization on N_2O release and CH_4 uptake of a well-drained topsoil demonstrated by a microcosom experiment. **Z. Pflanzenenahr. Bonenkd**. 159: 129-135.

Focht, D. D. and W. Verstraete. 1977. Biochemical ecology of nitrification and denitrification. **Ad. in microb Ecol**. 1: 135-214.

Freney, J. R, D. L. Chen, A. R. Mosier, I. J. Rochster, G. A. Constable and P. M. 1993. Use of nitrification inhibitors to increase fertilizer nitrogen recovery and lint yield of irrigated cotton**. Fert. Res**. 34: 37-44.

Freney, J. R., C. J. Smith and A. R. Mosier. 1992. Effect of a new nitrification inhibitor (waxt coated calcium carbide) on transformation and recovery of fertilizer nitrogen by irrigated wheat. **Fer. Res**. 32: 1-11.

Gillan, S. F., C. C. Somervelle, N. L. Lytte and M. B. Adams. 2001. Factors influencing spatial variability in nitrogen processing in nitrogen saturated soils. **The Scientific World** 1: 505-513

Gilmour, J. T. 1984. The effect of soil properties on nitrification and nitrification inhibition. **Soil Sci. Soc. Am.J**. 48: 1262-1266.

Goring, C.A.I 1962. Control of nitrification by 2-chloro-6-(trichloromethyl)pyridine. **Soil Sci.** 93: 211-218.

Gregory, M.W. 1998. **Mode of action of nitrification inhibitors**. Tektran .

Hart, C. S., J. M. Stark, E. A. Davidson and M. K. Firstone. 1994. Nitrogen mineralization, immobilization and nitrification. pp. 985-1011. *In*: R. W. weaver, S. Angle, P. Bottomley, D. Bezdiicek, S. Smith, A Tabatabia and A. Wollun (eds.). **Methods of soil analysis, part 2: Microbiology and biochemical properties**. SSSA Book series, no. 5. Madison, WI.

Hauck, R. D. 1980. Mode of action of nitrification inhibitors. pp. 19-32. *In*: J. J. Meisinger, , G. W. Randall and M. L. Vitosh (eds.). **Nitrification inhibitors potential and limitations**. ASSSA, Special publication. Madison, WI.

Hauck, R. D. 1984. Technological approaches to improving the efficiency of nitrogen fertilizer use by crop plants, pp. 551 – 560. In Ronald D. Hauck (ed.). I **Nitrogen in crop production**. ASA-CSSA-SSSA, Madison, WI.

Hayatsu, M. and N. Kosuge. 1993. Autotrophic nitrification in acid tea soils**. Soil Sci. Plant Nutr.** 39: 209-217.

Hendrickson, L. L. and D. R. Keeney. 1975. Effect of some physical and chemical factors on the rate of hydrolysis of nitrapyrin (N-serve). **Soil Biol Biochem**. 11: 47-50.

Hergert, G. W. and R. A. Wiese. 1980. Performance of nitrification inhibitors in the mid west. Pp. 89 – 106. *In*: Meisinger, J. J., G. W. Randall and M. L. Vitosh.1980. **Nitrification Inhibitors: Potential and Limitations**, Spec.Pub.38,129p. ASA and SSSA, Madison,WI.

Herlihy, M and W. Quirke. 1975. The persistence of 2-Chloro-(6 Trichloromethyl) Pyridine in soil. Commun. **Soil Sci. Plant. Anal**. 6: 513-520.

Hermansson, A., J. S. K., Backman, B. H. Svensson and P. E. Lindyren. 2004. Quantification of ammonia-oxidizing bacteria in limed and non-limed acid coniferous forest soil using real-time PCR. **Soil Biol. Biochem**. 36: 1935-1941.

Hoeft, R. G. 1984. Current status of nitrification inhibitor use in U. S. Agriculture, pp. 551-569. *In*: R. D. Hauck (ed.). **Nitrogen in crop production**: Proceedings of a symposium held 25-27 May 1982 Sheffield. ASA-CSSA-SSSA, Madison, WI.

Indian Institute of Spices Research. The secret to fine cuisine are fresh spices. http://www.harvestfields.ca/CookBooks/spice/cloves.htm, 2006.

Janesh, M. C. P. 1981. Species, condiments and medicinal plants in Ethiopia, their taxonomy and agricultural significance. Center for Agricultural publishing and documentation, The Netherlands.

Jarvis, S. C and D. Barraclough. 1991. Variation in mineral nitrogen content under grazed grassland sward**. Plant Soil**. 138: 177-188.

Jarvis, C. S., E. A. Stockdale, M. A. Shepherd and D. S. Powlson. 1996. Nitrogen mineralization in temperate agriculture soils. Process and measurements. **Adv. Agron**. 57: 187-233.

Jha, P.P., J. S. Singh and A. K. Kashyap. 1996. Dynamics of viable nitrifiers community and nutrient availability in dry tropical forest habitat as affected by cultivation and soil texture. **Plant Soil**. 138: 177-188.

Jenkinson, D. S., R. H. Fox and J. H. Lips. 1994. Interactions between fertilizer nitrogen and soil nitrogen. The so called “Priming” effect**. J. Soil. Sci**. 36: 425-444.

Johnson, J. W. 2002. Nitrification inhibitors potential use in Ohio. State University extension. Department of horticulture and crop science.

Justice, J. K. and R. L. Smith. 1962. Nitrification of ammonium sulphate in calcarious soils as influenced by combinations of moisture, temperature and levels of added nitrogen. **Soil Sci. Proc.** 246-250.

Keeney, D. K. 1980. Factors affecting the persistence and bioavailability of nitrification Inhibitors. Pp. 33-46. In J. J. Meisinger, G. W. Randall and M. L. Vitosh.1980. **Nitrification inhibitors: potential and limitations**, Spec.Pub.38,129p.ASA and SSSA, Madison,WI.

Keeney, D. R. and D. W. Nelson. 1982. Nitrogen: Inorganic forms, pp 648-654. In A. L. Page and D. R. Keeney (eds.). **Methods of soil analysis, part 2. Chemical and microbiological properties of soil analysis**. Agronomy Monograph. No. 9 ASA, Madison.

Kelsa, K., T. Yohannes and T. Bogale. 1992. Influence of fertilizer and its related management practice on maize grain yield in major maize producing areas of Ethiopia. In Benti, T. and J. K. Ramsom (eds.). **Proceedings of the first national maize workshop of Ethiopia**. 5-7 may 1992 , Addis Ababa, Ethiopia.

Knowles, R. 1999. Factors affecting the localization of nitrogen and methane in the soils. "http://plato.acadiau.ca/isme/Symposium02/knowles" . November 28, 2005.

Kreiting, J. P., T. M. Klien, N. J. Novick and M. Alexander. 1985. Nitrification characteristics

of nitrifying microorganisms in acid forest soil. **Soil Sci. Soc. Am. J.** 49: 1407-1410.

Kumar, K., S. Singh, S. N. Sharma and Y.S. Shivay. 2000. Relative efficiency and dicyandiamide-blended urea on mustard (*Brassica juncea*) varieties. **Indian J. Agron**. 45: 179-183.

Kotbe, F. 1985. **Medicinal plants in Libiya**. Arab encyclopidia house in Libiya

Lindsay, W. L and W. A. Novell. 1978. Development of a DTPA soil test for Zn, Fe, Mn and Cu. **Soil Sci. Soc. Am**. J. 42:421-428.

Mahli, S. S., and W. B. McGill. 1982. Nitrification in three Alberta soils. Effect of temperature, moisture and substrate concentration. **Soil. Biol. Biochem**. 14: 393-399

Malhi, S.S and M. Nyborg. 1982. An evaluation of carbon disulphide sulphur fertilizers as nitrification inhibitors. **Plant Soil**. 65: 203-218.

Mclean, E.O. 1982. Soil pH and lime requirement. Pp 199-224. In A. L. Page, R. H. Miller and D. R. Keeney (eds.) **: Methods of Soil Analysis part II**, 2nd ed ASA, Madison.

McCarty, G. W. and J. M. Bremner. 1989. Laboratory evaluation of dicyandiamide as as a soil nitrification inhibitor**. Commun. Soil Sci. Plant Anal**. 20 2049-2065

Meisinger, J. J., G. W. Randall and M. L. Vitosh.1980. **Nitrification inhibitors: Potential and Limitations**, Spec.Pub.38,129p. ASA and SSSA, Madison,WI.

Mintesinot, A. 1999. **Invitro antimicrobial activity of some traditional medicinal plants on food borne pathogens**. MSc. Thesis, Addis Ababa University, Ethiopia.

Mintesnot, A. and A. Mogessie. 1999. Assessment of the antibacterial activity of some traditional medicinal plants on food- borne pathogens. **Ethiop. J. Health Dev**. 3: 211-216.

Mobarry, B. K, M. Wagner, V. Urbain, B.E, Rittam, and D. A, Stahl. 1996. Phylogenic probe for analyzing abundance and spatial organization of nitrifying **Applied and environ. microb**. 62: 2156-2162.

Moore,A. and K Smiciklas . 1997. Linkages: lake Bloomington sustainable water program. Illinois State Department of Agriculture

Morill, L.G and Dawson, J. E. 1967. Pattern observed for the oxidation of ammonium to nitrate by soil organisms. **Soil.Sci. Soc. Am.proc.** 31: 750-60.

Myers, R. J. K. 1975. Temperature effect on ammonification and nitrification in tropical soils. **Soil Biol. Biochem**. 7: 83-86.

Nelson, D. W. and D. Hubner. 1980. Performance of nitrification inhibitors in the mid wast (east). Pp. 75-88. *In*: J. J. Meisinger, G. W. Randall and M. L. Vitosh.1980. **Nitrification inhibitors: potential and limitations**, Spec.Pub.38,129p. ASA and SSSA, Madison,WI.

Nelson, D. W and D. Hubner. 2001. Nitrification inhibitors for corn production. National corn handbook. Iowa State University.

Ockman, O. C and H. W. Olfs. 1998. Fertilizer, agronomy and N_2O. **Nutr. Cycl. Agroecosyst**. 52:167-170.

Olsen, R. S. and L. E. Sommers. 1982. Phosphorus. Pp. 403-427. *In*: A. L Page , R.H. Miller and D. R. Keeney (eds.**), Methods of Soil Analysis , Part I**I 2^{nd} eds. ASA, Madison.

Owens, L. B. 1987. Effect of nitrapyrin on nitrate movement from monolit in soil columns**. J. Environ. Qual**. 10: 308-310.

Paavolainen, L., Kutunen, V. and A. Smolander. 1998. Inhibition of nitrification in forest soil by monoterpenes**. Plant Soil**. 205: 147-154.

Paul, E. A. and Clark, F. E. 1989. **Soil microbiology and Biochemistry**. Academic Press, Sandiago. 340P.

Paulos D. 2001. Soil and water resource degradation factors affecting their productivity in Ethiopian high land agro ecosystems. In abstracts of international conference on the contemporary development issues in Ethiopia. CADPR,Western Michigan University.

Pochana, k. 1999. Study of Factors Affecting Simultaneous Nitrification and Denitrification (SND). Water Science. www.awmc.uq.edu.au/alumni/klang.html,. November 28, 2005.

Potash and Phosphate Institute. 1979. **Soil Fertility Manual.** Atlanta, Georgia.

Prasad, R. and F. J. Power 1997. **Soil Fertility Management for Sustainable Agriculture**. Lewis Publisher, New York. 356p.

Prasad, R. and F. J. Power 1995. Nitrification inhibitors for agriculture, health and the environment. **Adv. Agron**. 54: 233-278.

Prosser, J. I. 1989. Authotrophic nitrification in bacteria. **Adv. Microbial Physiol**. 30: 125-181.

Pulimin, O and A. Huxely. 1965. **Flowering Plants of Meditranean, Chattu and Windus**, London

Rao, D. L. N. and S. K. Ghai. 1986. Urease Inhibitors: Effect on wheat growth in an alkali soil**. Soil Biol. Biochm**. 18: 255-258.

Reddy, R. N. S. and R. Prasad. 1975. Studies on the mineralization of urea and nitrification inhibitors treated urea in soil. **J. Soil Sci**. 26: 305- 312.2.

Rendle, B. A. 1979. the classification of flowering plants. VII. Dicotyledons. Vikas publishing PVT LMTD, India.

Reynoldsvarga, J. S, D. D.Richeter and E. Bronemisza. 1994. Environmental impacts of nitrification and nitrate adsorption in fertilized Andisols in the valley of central Costa Rica . **Soil sci**.157:289-218.

Rice, E. L and S. K.Pancholy 1973. Inhibition of nitrification by climax ecosystem. II. Additional evidences and possible role of tannins. **Amer. J. Bot**.60: 691-702.

Rice, E. L and S. K.Pancholy 1974. Inhibition of nitrification by climax ecosystem. III. Inhibition other than tannin. **Amer. J. Bot**.61: 1095-1103.

Rowell, D. L. 1994. **Soil Science: Methods and Applications**. Longman Scientific and Technical, Singapore. 350p.

Sahrawat, K. L. and B.S. Parmar.1975. Alcohol extract of Neem (*Azadirzacta indica* L) seed as nitrification inhibitor. **Indian J. Soc. Soil Sci**. 23: 131-134.

Sahrawat, K. L. 1989. Effect of nitrification inhibitors on nitrogen transformation, other than nitrification, in soils**. Adv. Agron**. 42: 279-307.

Sahrawat, K. L., D. R. Keeney and S. S. Adams. 1987. Ability of nitrapyrin, dicyndiamide and acetylene to retard nitrification in mineral and organic soil. **Plant Soil**. 101: 179-182.

Sahrawat, K. L. 1980. On criteria for comparing the ability of compounds for retardation of nitrification in soil. **Plant Soil**. 55: 487-490.

Sahrawat, K. L. 1982. Nitrification in some tropical soils. **Plant Soil**. 65: 281-286.

Sahrawat, K. L. and S. K. Mukerjee and K. C. Gulati. 1977. Nitrification inhibitors. I. Studies with karanajin, a furano flaviniod from karanaja *(Pongamia glabra* vet.). **Plant Soil**. 47:27-36.

Sartachandra, S.U. 1978. Nitrification activities of some New Zealand soils and the effect of some clay types on nitrification. **J. of Agric. Res**. 21: 615-62.

SAS Institute. 1989> SAS/STAT; user's guide version 6.2. 4th edition, SAS Institute, Cary,NC.

Schmidt, E. L. 1982. Nitrification in soil. In F. J. Stevenson (ed.). Nitrogen in Agricultural soils. **Agron**. 22: 253-288.

Schmidt, E. L. and L. W. Belser. 1994. Authotrophic nitrifying bacteria, pp . 159-177. *In*: R. W. weaver, S. Angle, P. Bottomley, D. Bezdiicek, S. Smith, A Tabatabia and A. Wollun (eds.). **Methods of Soil Analysis, part 2. Microbiology and Biochemical Properties**. SSSA Book series, no. 5. Madison, WI. Sci. Plant Nutr. 39: 209- 217.

Shaviv, A.2001. Advance in controlled-release fertilizers. **Ad. Agron**. 71: 1-49

Sing , M. K., S. K. Verma and R.Thankur. 1999. Efficacy of slow release nitrogen fertilizers to rice (Oryza sativa) and their residual effect on wheat (*Triticum aesativum*). **Indian J.**

Agron. 44: 102-104.

Sprent, J. I. 1987. The ecology of nitrogen cycle. Cambridge University Press.

Ste-Marie, C. and D. Pare. 1999. Soil, pH and N availability effect on net nitrification in the forest floor of range of boreal forest and stand. **Soil Biol and Biochem**. 31: 1579-1589.

Strong, D. T., P. W. G. Stale and K. R. Helyar. 1999. The influence of soil matrix on nitrogen mineralization and nitrification. III. Predictive utility of traditional variable and process location with in the pore system. **Aust. J. of Soil Res**. 37: 137-149.

Sue, E. and A. Zemede. 1992. **Plants used in Africa as practiced in Ethiopia and Uganda Botany 200**: East and Central Africa Monograph series number, NAPRECA, Addis Ababa University, Addis Ababa.

Subba Rao, N. S. 1993. **Biofertilizers in agriculture and forestry**. 3rd ed., Oxford and IBH Publishing Co. PVT., New Delhi. 242p.

Sumner, M. 1999. **Soil Science. 2nd ed. Hand Book of Soil Science**. CRC Press, London.

Tate, R. L. 2000. **Soil Biology.** 2nd eds. John Wiley and Sons, New York. 508p.

Taye, B., H. Yesuf, S. Sahlemedhin, G. Amanuel, H. Hassena, D. G. Tanner, T. Tesfaye and G. Takele. 2002. **Optimizing Fertilizer Use in Ethiopia: Correlation of Soil Analysis with Fertilizer Response in Hetosa Woreda, Arsi zone**. Addis Ababa, Sasakawa-Global 2000.

Theron, J. J. 1951. The influence of plants on the mineraliztion of nitrogen and maintenance of organic matter in soil. **J. Agric. Sci.** 41: 289-289

Thomas, G. W. 1982. Exchangeable cation. P.159. *In*: A. L. Page, R. H. Miller and D. R. Keeney (eds.) **Methods of Soil, analysis**, part II 2nd edition ASA, Madison.

Thompson, W. E., W. R Raun and G. V., Johnson.2000. Winter wheat fertilizer nitrogen use efficiency in rain and forage production. **J. of Plant Nutr**. 23:1505-1516.

Tisdale, S. T., W. L. Nelson and J. D. Beaton. 1985. **Soil Fertility and Fertilizers**. 4th ed. Macmillan Publishing Company, New York. 499p.

Touchton, J. T., R. C. Hoeft, L. F. Welch, W. L. Argyilan. 1979. Loss of nitrapyrin from soils as affected by pH and temperature**. Agron. J**. 71: 865-869.

Troelstra, S. R., R. R. Wagnaar and De W. Boer. 1990. Nitrification in Dutch heathland soils: I. General characteristics and nitrification in undisturbed soil cores. **Plant soil**. 127: 179-192.

Turner, G. O. and C.A.I. Goring .1966. N-SERVE- A status report- **Down Earth.** 22: 19-25.

UNDP-Emergency unit for Ethiopia. 1995. Country information in brief. Food and Agricultural Organization of the United Nations, Draft, June 1995.

Vismeier, K. 1980. Dicyandiamidabbau in boden in abhangigkeit von der temperatur. **Z. Planzen Bodenk**. 143: 113-118.

Vitosh, M. L and L. W. Jacobs. 1996. Nitrification Inhibitors: Nutrient Management to Protect Water Quality, Extension Bulletin,Michigan State University . web1.msue.msu.edu/imp/modf1/06109717.html, Retrieved. 17 November 2005.

Walkley, A and I.A. Black . 1934. An examination of the digitijareff method for determining soil organic matter and a proposed modification of chromic acid titration method. **Soil Sci**. 153: 100-107.

Wassie, H., T. Mala and V. Verasan. 2006. **Screening of Ethiopian traditional medicinal herbs for their nitrification inhibiting ability**. Ku. J. in press

Wassie, H., T. Mala, Y. Osotsapar and V. Verasan. 2004. Investigation on the nitrification potential of some soils occurring in southern and central Ethiopia. **Kamphaengsean Acad. J**. 2: 111-125.

Weier, K.L and J. W. Gilliam.1986. Effect of acidity on nitrogen mineralization and nitrification in atlantic coastal plain soils. **Soc Sci. Soc.Am. J**. 50:1210-1214.

Wick, B., R. F. Kune and P. I. G. Vlek. 1998. Soil microbiological parameters as indicators of soil quality under improved fallow management system in southern Nigeria. **Plant Soil**. 202: 97-107.

Woldemichael, K. 1987. **A glossary of Ethiopian Plant Names**, 4th ed. Addis Ababa 45p.

Woldendore, P. J. W. and H. J. Laanbbroek.1989. Activity of nitrifies in relation to Nitrogen nutrition of plants in natural ecosystems**. Plant Soil**.115: 217- 228.

Zemenchik, R. A. 2002. Nitrogen use efficiency and apparent recovery of Kentucky blue grass , Smooth bromgrass and orchard grass. **Agron. J**. 94: 421-428

Yesuf, H. 2000. The effect of rate and time of N application on its uptake by bread wheat varieties and soil characteristics of farmers' field. **Ethiopian J. of Natural Resources**. 2: 137-150.

Yesuf, A., O. Vancleemput. 2001. The effect of urease inhibitors and their mixture on the mineral N content of sandy loam soil after urea application, pp. 23-34. *In:* W. Chekol and E. Mersha (eds.). **Nutrient management for improved soil/crop Productivity in Ethiopian agriculture**. Proceedings of the 5th conference of ESSS, March 30-31, 2000, Addis Ababa

Yohannes, U . 1994. **The effect of nitrogen, phosphorus, potassium, and sulphur on the yield and yield components of Ensete (*Ensete ventricosum* W.) in southwest of Ethiopia**. Ph.D. dissertation, Germany.