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Screening of Ethiopian Traditional Medicinal Herbs for their Nitrification Inhibiting Ability

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ABSTRACT

Nitrification is the main cause of reduced N-use efficiency and yield of crops. Blending nitrogenous fertilizer with commercial or herb-based inhibitors is one of the means of increasing N-use efficiency. The objective of this study was to screen Ethiopian traditional medicinal herbs along with Neem (none traditional Ethiopian medicinal plant) and commercial inhibitors for their nitrification inhibiting ability. Soil samples were collected from Awassa Agricultural Research Center, Ethiopia. One hundred gram of processed soil was transferred to 250 ml capacity cups to which alcohol extracts of 11 herbs at 1% rate of dry soil were added separately. N-seve ([2-chloro-6 (trichloromethyl) pyridine]) and Dicyandiamide (DCD) were added at a rate of 2 and 100μg/g of soil respectively. The cups were also amended with 50 mg of ammonium sulphate and the moisture was maintained at 60% water holding capacity (WHC) and incubated for various period at 25°C. At the end of each period, samples were analyzed for pH, NH_4-N and NO_3-N. The result revealed that 72% of the tested herbs and commercial inhibitors conserved significantly high amount of NH_4-N, an indication of inhibition of nitrification, at the end of 2nd week. But from among Ethiopian medicinal herbs, only *Artemis afra*, *Echinops spp* and *Eugenia caryophyllata* inhibited nitrification at the end of 3rd week. Averaged over two incubation periods, these herbs inhibited nitrification by 33, 37 and 64% respectively. *Eugenia caryophyllata* performed as effective as neem but none of the herbs out performed commercial inhibitors. Both medicinal herbs and commercial inhibitors prevented the soil from the acidification of the soil. There was a decline in inhibition percentage of all inhibitors with time but it was more drastic for herbs than commercial inhibitors. It is concluded that 3 Ethiopian medicinal herbs were identified as nitrification inhibitors. Thus, their extracts could be blended with ammonium fertilizers for increasing N-use efficiency of crops.

Key words: medicinal herbs, nitrification, percentage inhibition ability

INTRODUCTION

Nitrogen is by far the most important nutrients required by crops or plants for their growth and development. However, it is deficient in most Ethiopian soils which resulting in limiting crop production. Traditional methods of soil N restoration methods such as fallowing, crop rotation with legumes and incorporation of manure are abandoned due to shortage of land which in turn is caused by ever increasing human population.

In Ethiopia, to overcome N deficiency and increase crop production, application of N-containing chemical fertilizers has been started since 1971. Its consumption increased from 947

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metric tons in 1971 to 446 000 tons in 2002 (Taye *et al*., 2002). Appreciable increases in the yields of several crops have been obtained due to this practice. However, the costs of chemical fertilizers are increasing from time to time becoming unaffordable to subsistent farmers. More over, the fertilizer use efficiency of crops is very low. For instance, in USA, maximum of 50% of the applied N is utilized by corn (Nelson and Huber, 2001). The situation is very severe in the tropics where only 25 to 40 % of the applied N is utilized by crops in a season (Sahrawat and Mukerjee, 1977).

Nitrification is the main cause of reduced N use efficiency of crops, because it converts ammonium to nitrate, which is freely mobile and subjected to losses via leaching and denitrification (Jarvis *et al*., 1996). Thus, the control of nitrification in the soil is indispensable to increase N-use efficiency (Hauck, 1984).

There are several methods of reducing N loss and improving N-use efficiency of crops. Such methods include proper time and methods of application of fertilizers, use of slow release fertilizers and use of nitrification inhibitors (Hauck, 1984).

Nitrification inhibitors are chemical compounds that kill ammonia oxidizing bacteria or interfere with their metabolism. As a result N is maintained in the form of NH_4 ion which is less subject to losses (Hauck, 1984). To date, there are several compounds tested and produced commercially worldwide. Among them N-serve [2-chloro-6 (trichloromethyl) pyridine] and dicyandiamide (DCD) are by far the most widely used for nitrification inhibitors (Prasad and Power, 1995). Details on various aspects of these inhibitors could be found in Meisinger *et al*. (1980).

Although, there are variations among soil types and crops with respects to effectiveness of nitrification inhibitors, appreciable increase in the yields and N-use efficiencies of several crops were obtained by blending N-fertilizers with nitrification inhibitors (Prasad and Power, 1995).

There are also nitrification inhibitors of plant origin. Paavolainen *et al.* (1998) reported that monoterpenes that are produced by roots of Norway spurce (*Picea abies* L.) inhibits nitrification. Neem (*Azadirachta indica*) cake was found to inhibit nitrification effectively both in the laboratory and greenhouse (Sahrawat and Parmer, 1975). Nitrification inhibition property was also identified in Karanaja (*Pongamia glabra)* (Sahrawat and Mukerjee, 1977) and pyrithrum flower. The exploitation of such plants as inhibitors is important, as commercial inhibitors are expensive for developing countries like

In this regard, there are several Ethiopian traditional medicinal herbs with anti-microbial property against human pathogenic bacteria (Mintesnot and Mogessie, 1999). But they have never been tested for their nitrification inhibiting ability. The objective of this study was therefore to investigate the nitrification inhibiting ability of some Ethiopian medicinal herbs in comparison with commercial inhibitors.

MATERIALS AND METHODS

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 1.

The samples were thoroughly mixed, air-dried and milled to pass 4 mm sieve. One hundred gram of processed soil was transferred into 250ml capacity plastic cups.

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 2. 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 °C.

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 °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).

Table 1 Chemical characteristics of soil used in the experiment.

pH	TN (%)	OM (%)	BS (%)	Na	K	Ca	Mg	CEC	P	Fe	Mn	Zn	Cu
				cmol /kg					mg /kg				
7.6	0.15	3.4	68	0.4	5.53	4.19	1.03	21.2	46	18	26	6.4	0.14

Table 2 Description of some of traditional medicinal herbs screened as nitrification inhibitors (Woldemichael, 1987).

Common name	Scientific name	Family name	Parts use
Wormwood	*Artemisa 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

The percentage 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. Data on NH_4-N and NO_3 -N were subjected to statistical analysis using SAS software version 6.2. When found significant, means were ranked using Duncan's multiple range tests.

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.

RESULTS AND DISCUSSION

Preliminary screening

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 3. 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

Table 3 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 of soil and incubated at 25°C.

Medicinal herbs/	Incubation periods (weeks)					
	2			3		
Inhibitors	pH	NH_4-N	NO_3-N	pH	NH_4-N	NO_3-N
Artemisa afra	6.65	89.7c*	43.5cd	6.65	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	6.56	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	6.56	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	7.13	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	7.53	88.7b	38d
N-serve	7.95	137.1ab	16.8f	7.90	110.9a	29.8ed
DCD	7.03	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 colums followed by the different letters are significantly diferrent from each other at P< 0.05 according to DMRT

Eugenia caryophyllata, *Echinops spp.*, *Artemisa afra* and neem (*Azadiracta indica*). These herbs were previously reported to have antibacterial 9killing 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. The occurrence of plant extracts and their exudates as nitrification inhibitor are also reported by several authors (Pacncholy, 1973; Sahrawat and Mukerjee, 1977; Rice and 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).

According to Prasad and Power (1995), epinibin, nibin, desacetylnimbin, salanin, desacetylacinin and azadiractin collectivelly known 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 varied 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 4. Of all herbs only *Artemisa afra*, *Echinops spp*., *Eugenia caryophyllata* and Neem 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.

Secondary screening

The effect of alcohol extracts of *Artemisa afra, Echinops* sp. and *Eugenia caryphyllata* and commercial inhibitors on NH_4-N and NO_3-N contents of soil is presented in Table 5. These 3 herbs conserved significantly ($P<0.01$) high amount of NH_4-N up to 4th week as compared to the control. *Eugnia carophyllata* was the most effective inhibitor followed by *Echinops spp* and A*rtemisa 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 6. The highest degree of inhibition among 3 herbs was achieved from *Eugenia caryophyllata*., followed by *Echinops sp*. and the least was *Artemisa 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 *Artemisa 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.

Effect of nitrification inhibitors on soil pH

The result of effects of herbal and

commercial inhibitors on soil pH which was monitored in the secondary screening experiment is shown in Table 7. 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 inhbitors 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 nitrifcation inhibitors in addition to inhibiting nitrification, are also prevented the soil from acidification.

Table 4 Nitrification inhibition percentage by Ethiopian traditional medicinal herbs.

Medicinal herbs/Inhibitors	% Inhibition		
	Incubation periods (weeks)		
	2	3	Mean
Artemisa 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 colums followed by the different letters are significantly diferrent from each other at P< 0.05 according to DMRT.

Table 5 Effect of Ethiopian medicinal herbs on the NH_4-N and NO_3-N content in Awassasoil (Eutric fluvisol) amended with ammonium sulphate and incubated for various periods of time.

Medicinal herbs/Inhibitors	Incubation periods (weeks)					
	2	3	4	2	3	4
	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 with in colums followed by the different letters are significantly differrent from each other at P< 0.05 according to DMRT.

Table 6 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 colums followed by the different letters are significantly different from each other at P< 0.05 according to DMRT.

Table 7 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°C.

Medicinal herbs/	Incubation periods (weeks)		
Inhibitors	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
Contro	6.5	6.7	6.5
Mean	7.0	6.97	6.76
SE±	0.13	0.13	0.07

CONCLUSION

In conclusion, 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 4 weeks of incubation. The highest percentage nitrification inhibion was achieved at the end of 2 weeks of incubation and the least at the end of 4 weeks of incubation. *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 out shined the commercial inhibitors. Application of both heral 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. They are particulary applicable to subsistent farmers who can not 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. Thus, These inhibitors could be imported are used by large scale private farmers which can afford the incurred expenes.

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