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The Effect of Some Thai Medicinal Herb Extracts on Nitrification Inhibition

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Abstract

This study aimed to determine the effect on nitrification inhibition of some Thai medicinal herb extracts. The experimental design was completely randomized design, consisted 33 treatments with 4 replications. The experiments were performed in a laboratory, using surface soil of Yang Talat soil series as media. In each treatment, soil sample (100 g) was mixed thoroughly with 1 ml of herbal extract and 50 mg kg^{-1} of ammonium sulphate. The mixture was then incubated at ambient temperature. Ammonium oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), ammonium-N (NH_4^+-N), nitrate-N (NO_3^--N), and soil pH were determined at week 1 to 4 after incubation. The results showed that the numbers of AOB in soil sample mixed with galanga stem decreased during the first 2 weeks. It was obvious that after the first week of incubation, the activity of NOB in soil samples mixed with extracts of ringworm bush leaf, heart- leaved moonseed stem, mangosteen fruit, kariyat leaf and galanga rhizome was suppressed. Soil samples containing herbal extracts had higher concentraions of NH_4^+-N and lower concentraions of NO_3^--N than those of control. The highest amount of NH_4^+-N was found in clove flower treated soil during the first 2 weeks of incubation. Kariyat leaf treated and cinnamon bark treated soil samples contained the highest amount of NH_4^+-N in weeks 3 and 4, respectively. Samples with extracts of ringworm bush leaf, mangosteen fruit, Thai copper pod leaf, Indian mulberry leaf, lemon grass leaf, bitter cucumber fruit, egg woman stem, fingerroot stem, fingerroot rhizome and hog plum leaf contained the lowest amount of NO_3^--N during the first 3 weeks. The concentraion of NO_3^--N in heart- leaved moonseed stem treated soil was the lowest in the last 3 weeks. The highest ratio of NH_4^+-N (100 %) and the lowest ratio of NO_3^--N (0.0 %) to inorganic N were found in samples with extracts of ringworm bush leaf, mangosteen fruit, Thai copper pod leaf, Indian mulberry leaf, lemon grass leaf, bitter cucumber fruit, egg woman stem, fingerroot stem, fingerroot rhizome, hog plum leaf and heart-leaved moonseed stem for 3 weeks. At early stages of incubation, low pH of herbal soil samples were observed. The pH of those samples, however, increased over time.

Keywords: ammonium nitrogen, ammonium oxidizing bacteria (AOB), herb extracts, nitrification inhibition, nitrate nitrogen, nitrite oxidizing bacteria (NOB)

1. Introduction

The application of chemical fertilizer increases production efficiency and provides better quality of products in agricultural activities. However, fertilizer consumption has increased exponentially throughout theworld, causing serious environmental problems. Some fertilizer may increase the accumulation of heavy metals in soil and plant systems and leads to water, soil and air pollution (Savci, 2012) that can be absorbed by plants and enter the food chain. Nitrogen is one of the most important nutrients required by crops or plants for their growth and development. The addition of nitrogen can affect microbial biomass, activity, and species composition (Crecchio et al., 2001; Jumadi et al., 2005; Sarathchandra et al., 2001). Nowadays, human beings are aware of the use nitrogen in larger quantities than plant need to be harmful to the environment. Nitrogen fertilizers break down into nitrates and travel easily through the soil. Because it is water-soluble and can remain in ground water for decades, the addition of nitrogen over the years results in an accumulative effect (Savci, 2012). Losses of applied nitrogen as leachable nitrate (NO_3^-) and as emissions of nitrogenous gases (N_2, N_2O, NO_x) can occur from both nitrification and subsequent denitrification. The nitrification process is primarily accomplished by two groups of autotrophic nitrifying bacteria that can build organic molecules using energy obtained from inorganic sources, in this case ammonia or nitrite. In the first step of nitrification, ammonia is oxidized to nitrite by ammonia oxidizing bacteria (AOB), and then to nitrate by nitrite oxidizing bacteria (NOB) in the second stage. Thereafter, nitrate is reduced to nitrite, and then to nitrogen gas (N_2) by denitrification stages (Zeng, et al., 2014). These gases contribute to the greenhouses effect. One method of reducing N losses from nitrification is to reduce the rate of nitrification by inhibiting the nitrifying bacteria, and increase the rate of plant uptake of ammonium (NH_4^+). This can be achieved by using nitrification inhibitors (NI) (Weiske et al., 2001; Maeda et al., 2003; Di & Cameron, 2005). The NI played important roles in enhancing yields and reducing N_2O emissions from the vegetable ecosystem (Zhang et al., 2015). The inhibitors have shown to decrease leaching from urea and ammonium-based fertilizers (Di et al., 2007; Monaghan et al., 2009). It can decrease NO_3^- concentrations in the leaching and reduce the potential accumulative NO_3^- loss in agricultural land (Weiske et al., 2001; Yu et al., 2007; Hua et al., 2008). Nitrification inhibitors, including commercial products like N-Serve or nitrapyrin, dicyandiamide (DCD) (Amberger, 1989) and 3, 4-dimethylpyrazole phosphate (DMPP) (Zerulla et al., 2001) inhibit ammonia monoxygenase enzyme and prevent the conversion of NH_4^+ to NO_3^-, implying specificity in their mode of action (Prasad, 2009). The high cost of commercial inhibitors is another factor that limits its large scale use in agriculture, where profit margins are relatively small and the return from investment is uncertain. Commercial nitrification inhibitors also have limited availability and have detrimental effects on the environment (Upadhyay et al., 2011). In fact, suppression of soil nitrification has been observed in some natural ecosystems (natural nitrification inhibition). It helps conserving soil N and improving N status through lower formation of NO_3^- (Lata et al., 2004; Subba Rao et al., 2006). The use of such plants as nitrification inhibitors, which are eco-friendly and biodegradable, therefore hold considerable potential. Several experimental studies have indicated that some plants and their by-products are able to inhibit nitrification (Prasad et al., 1971; Lata et al., 2004; Patra et al., 2006; 2009; Kiran & Patra, 2002; Kiran et al., 2003; Haile et al., 2006). It has been reported that *Brachiaria humidicola* (Rendle) Schweick, karanj (*Pongamia glabra* Vent.), sweet sorghum (*Sorghum bicolor* L.), neem (*Azadirachta indica*), tea (*Camellia sinensis*), linseed oil (*Linum usitatissimum* L.), mahuwa (*Madhuca latifolia*), *Pyrethrum* spp., *Artemisia annua* L., mint (*Mentha spicata* L.), *Artemis afra*, *Echinops* spp and *Eugenia caryophyllata* are important sources of natural nitrification inhibitors (NNI) (Upadhyay et al., 2011). This experiment focused on highlighting the use of NNI in order to offer low cost plantation for farmers. The objective of this study was to investigate the nitrification inhibiting ability of various Thai medicinal herb extracts in surface soil of Yang Talat soil series.

2. Materials and Methods

The study was performed in a laboratory. The experimental design was completely randomized design, consisted of 33 treatments with 4 replications: 1) control 2) banana fruit; *Musa sapientum* L., 3) bird's eye chilli fruit; *Capsicum frutescens* L., 4) bitter cucumber fruit; *Momordica charantia* L., 5) bitter melon fruit 6) cinnamon *Cinnamonum* sp., 7) clove flower; *Syzygium aromaticum* (L.) Merr. & L. M. Perry, 8) coffee ground; *Coffea arabica* L., 9) egg woman stem; *Phyllanthus amarus* Schum & Thonn., 10) fingerroot rhizome; *Boesenbergia rotunda* (L.) Mansf., 11) fingerroot stem 12) galanga leaf; *Alpinia galanga* (L.) Willd., 13) galanga rhizome 14) galanga stem 15) ginger rhizome; *Zingiber officinale,* 16) guava leaf; *Psidium guajava* L., 17) heart-leaved moonseed stem; *Tinospora crispa* (L.) Miers ex Hook. f. & Thomson, 18) hog plum leaf; *Spondias pinnata* Kurz. 19) holy basil leaf; *Ocimum sanctum* L., 20) Indian mulberry leaf; *Morinda citrifolia* L., 21) kaffir lime leaf; *Citrus hystrix* DC., 22) kariyat leaf; *Andrographis paniculata* (Burm.f.) Wall.ex Nees, 23) lemon grass leaf; *Cymbopogon citratus* Stapf., 24) mangosteen fruit; *Garcinia mangostana* L., 25) pomegranate leaf; *Punica granatum* L., 26) ringworm bush leaf; *Cassia alata* (L.) Roxb., 27) Siamese neem leaf; *Azadirachta indica* A. Juss. var. siamensis Valeton, 28) Siamese neem seed 29) sugar apple leaf; *Annona squamosa* L., 30) sweet basil leaf; *Ocimum basilicum* L., 31) Thai copper pod leaf; *Senna siamea* (Lam.) Irwin & Barneby, 32) turmaric rhizome; *Curcuma longa* L., and 33) white pepper seed; *Piper nigrum* L.

2.1 Preparation of Herbal Extracts

The plant materials were cut in smaller pieces before drying at 60 ºC in hot air oven for 2 days. The dried materials were ground into 1 mm particle size using an electric grinder. The dried plant (1 g) was mixed with 10 ml of ethanol and the mixture was shaken for 20 min. The mixture was then filtered through a filter paper (Whatmann No. 1). The filtrate was evaporated to dryness and the dried residue was re-dissolved in 5 ml at a temperature 30 °C. (Sharma & Patel, 2009; Springfield et al., 2005)

2.2 Incubation of Herb Extracts with Soil

For each treatment, 100 g surface soil of Yang Talat Soil Series (pH = 5.2, NH_4^+-N = 45.8 mg/kg, NO_3^--N = 0.9 mg/kg) was placed in a 250 ml plastic cup. Ten ml of 1 % w/v ammonium sulphate was added to the cup. Herbal extracts (1ml) was added to the soil and moisture content of the soil mix was adjusted to 50-60 % WHC. The cup was then loosely capped and incubated at ambient temperature (25±2 °C). The experiment was performed in 4 replicates.

2.3 Data Collection

On a weekly basis, all samples were determined for the numbers of nitrifying bacteria using plate count method in NOB ($NaNO_2$, 1.0 g; Na_2CO_3, 1.0 g; NaH_2PO_4, 0.25 g; $MnSO_4.4H_2O$, 0.01 g; $MgSO_4.7H_2O$, 0.03 g; K_2HPO_4, 0.75 g; agar, 15 - 18 g; pH = 7.2 in 1,000 ml, pasteurized at 121°C for 30 min) and AOB($(NH_4)_2SO_4$, 1 g;$CaCl_2$, 1.5 g; $FeSO_4.7H_2O$, 0.03 g; K_2HPO_4, 1.0 g; NaCl, 0.3 g, $MgSO_4.7SO_4$, 0.03 g; agar, 16 - 20 g; PH = 7.0–7.2 in 1,000 ml, sterilized at 121°C for 30 min. (Wang Fang et al., 2007)). The bacterial population were presented in Log number. The concentrations of NH_4^+-N, and NO_3^--N, as well as pH values were analyzed using the procedures described by Aaronson (1971) and Alexander & Clark (1994). The percentage ratios of NH_4^+-N and NO_3^--N: inorganic N in the soil mixed with the herbal extracts was calculated as follows:

$$\% NH_4^+\text{-}N = \frac{NH_4^+\text{-}N}{(NH_4^+\text{-}N + NO_3^-\text{-}N)} \times 100$$

$$\% NO_3^-\text{-}N = \frac{NO_3^-\text{-}N}{(NH_4^+\text{-}N + NO_3^-\text{-}N)} \times 100$$

Where, NH_4^+-N is the total amount of ammonium nitrogen and NO_3^--N is the total amount of nitrate nitrogen in soil.

2.4 Statistic Analysis

One-way ANOVA was used for data analysis. Differences between mean values were subsequently analyzed by Duncan's new multiple range test.

3. Results

3.1 The Numbers of Ammonium Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB) in the Soil

The numbers of AOB in the soil mixed with Thai medicinal herb extracts were significantly different at 1-3 weeks (table 1). It was found that soil sample with white pepper seed extract contained the highest of AOB but the number was not significant different compared to other treatments. No AOB was detected in the samples containing extracts of holy basil leaf, galanga stem and lemon grass leaf at first week. Soil sample with clove flower extract had the highest of AOB, which did not differ from other treatments. No AOB was found in samples with galanga stem and bitter melon fruit extracts at 2 weeks. In week 3, turmaric rhizome-soil mixture had the highest of AOB, which was not significantly different from other treatments. No AOB was detected in the samples mixed with extracts of sugar apple leaf, egg woman stem, fingerroot stem, fingerroot rhizome and kaffir lime leaf. In week 4, the numbers of AOB in all treatments did not different. The amount of NOB in the soil mixed with Thai medicinal herb extracts were significantly different at 1 and 4 weeks (table 1). Soil sample with kariyat leaf extract showed the highest of NOB, which was not significantly different in comparison to those of the samples treated with ringworm bush leaf, Siamese neem seed, white pepper seed, pomegranate leaf, ginger rhizome, mangosteen fruit, Thai copper pod leaf, Siamese neem leaf, guava leaf, banana fruit, turmeric rhizome, cinnamon bark, sweet basil leaf, fingerroot stem and hog plum leaf. Furthermore, no NOB was found in the soil samples mixed with extracts of clove flower, holy basil leaf, bird's eye chilli fruit, galanga stem, galanga leaf, bitter cucumber fruit, egg woman stem and control after the first week of incubation. In week 2 and 3, the numbers of NOB in all treatments were not significantly different. In week 4, the numbers of NOB in each treatment were significantly different. Soil sample containing clove flower extract had the highest of NOB which was not significantly different with those of the samples containing coffee ground and turmeric rhizome extract. No NOB was found in control, and soil samples mixed with extracts of ringworm bush leaf, heart-leaved moonseed stem, mangosteen fruit, thai copper pod leaf, kariyat leaf, galanga rhizome, sugar apple leaf, bird's eye chilli fruit, cinnamon bark and galanga stem.

Table 1. The numbers of ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in soil samples supplemented with Thai medicinal herb extracts after incubation

Medicinal Herbs	Log number of AOB				Log number of NOB			
	week 1	week 2	week 3	week 4	week 1	week 2	week 3	week 4
Control	4.4 ab	5.2 ab	5.0 a	4.4	0.0 e	1.0	2.6	0.0 d
Banana fruit	2.1 ab	4.9 ab	4.6 a	5.0	3.5 abc	0.0	0.0	0.8 cd
Bird's eye Chilli fruit	2.0 ab	2.3 abc	2.0 ab	4.6	0.0 e	0.0	0.8	0.0 d
Bitter Cucumber fruit	1.3 ab	1.3 abc	1.3 ab	5.3	0.0 e	0.9	0.8	0.8 cd
Bitter Melon fruit	1.0 ab	0.0 c	1.0 ab	3.9	0.8 de	0.0	1.0	1.5 bcd
Cinnamon bark	2.3 ab	4.9 ab	3.6 ab	5.0	2.1 a-d	3.8	1.5	0.0 d
Clove flower	2.6 ab	5.7 a	4.5 a	5.4	0.0 e	1.7	1.5	4.1 a
Coffee ground	3.3 ab	4.3 abc	2.3 ab	4.8	0.8 de	0.8	0.0	2.8 abc
Egg Woman stem	1.4 ab	2.5 abc	0.0 b	5.1	0.0 e	0.8	0.0	0.8 cd
Fingerroot rhizome	1.0 ab	2.3 abc	0.0 b	4.8	1.6 cde	0.8	0.8	0.8 cd
Fingerroot stem	1.0 1	3.2 abc	0.0 b	5.0	3.4 abc	1.6	0.0	1.5 bcd
Galanga leaf	3.0 ab	3.4 abc	3.6 ab	4.9	0.0 e	1.8	0.0	0.7 cd
Galanga rhizome	4.4 ab	1.0 bc	3.0 ab	5.3	1.7 b-e	0.0	0.0	0.0 d
Galanga stem	0.0 b	0.0 c	4.6 a	5.0	0.0 e	0.0	1.5	0.0 d
Ginger rhizome	2.1 ab	4.1 abc	1.3 ab	5.3	3.6 abc	0.0	0.0	1.5 bcd
Guava leaf	3.1 ab	1.2 abc	4.7 a	5.4	3.0 abc	0.0	0.0	0.8 cd
Heart-leaved moonseed stem	4.0 ab	2.6 abc	4.2 ab	5.3	1.7 b-e	0.0	0.0	0.0 d
Hog Plum leaf	2.2 ab	3.6 abc	1.3 ab	5.1	2.3 a-d	0.8	0.0	0.8 cd
Holy basil leaf	0.0 b	1.0 bc	0.9 ab	5.4	0.0 e	0.8	0.8	0.8 cd
Indian Mulberry leaf	2.1 ab	2.1 abc	3.2 ab	5.3	0.8 de	1.8	0.8	0.8 cd
Kaffir lime leaf	2.7 ab	3.4 abc	0.0 b	5.3	0.8 de	0.8	0.8	0.7 cd
Kariyat leaf	3.4 ab	4.0 abc	3.6 ab	4.9	3.8 a	0.0	0.0	0.0 d
Lemon Grass leaf	0.0 b	2.6 abc	4.0 ab	5.4	0.8 de	0.8	2.3	0.8 cd
Mangosteen fruit	1.0 ab	4.5 abc	5.0 a	5.4	2.3 a-d	0.0	0.0	0.0 d
Pomegranate leaf	3.0 ab	3.1 abc	4.9 a	5.5	3.5 abc	0.8	0.0	0.8 cd
Ringworm Bush leaf	3.2 ab	4.4 abc	2.7 ab	5.4	3.7 ab	0.8	0.0	0.0 d
Siamese neem leaf	4.5 ab	2.4 abc	4.3 ab	5.0	3.4 abc	0.8	0.0	0.8 cd
Siamese neem seed	3.2 ab	5.3 ab	4.5 a	3.8	3.6 ab	0.0	0.0	0.8 cd
Sugar Apple leaf	2.1 ab	2.3 abc	0.0 b	5.3	0.8 de	0.0	0.8	0.0 d
Sweet basil leaf	2.1 ab	2.4 abc	3.7 ab	5.2	1.8 a-e	0.8	0.0	0.7 cd
Thai copper pod leaf	3.5 ab	5.3 ab	3.5 ab	5.4	3.4 abc	1.5	0.8	0.0 d
Turmaric rhizome	1.0 ab	2.4 abc	5.1 a	4.9	2.8 abc	1.6	0.8	3.0 ab
White pepper seed	4.8 a	4.8 ab	2.9 ab	5.3	3.3 abc	1.5	1.0	1.5 bcd
p-value	0.0	0.0	0.0	0.2	0.0	0.1	0.1	0.0
CV (%)	9.7	8.7	9.1	3.8	9.9	13.5	14.8	13.2

Note. Mean values in each column followed by the same letter are not significantly different by DMRT at $P \leq .05$.

3.2 Concentration of Ammonium Nitrogen (NH_4^+-N) in the Soil

The amount of NH_4^+-N in the soil samples supplemented with herbal extracts showed a significant difference (table 2). Addition of clove flower extract to the soil resulted in the highest amount of NH_4^+-N (233.4 mg/kg).

Such concentration, however, was not significantly different to those found in samples supplemented with cinnamon bark extract (219.9 mg/kg), banana fruit extract (197.8 mg/kg) and galanga leaf extract (197.1 mg/kg). The amount of NH_4^+-N in control treatment was the lowest (134.1 mg/kg) in the first week. In week 2, the amount of NH_4^+-N in clove flower treated soil remained the highest (131.4 mg/kg), but this did not differ from that of cinnamon bark treated soil (122.1 mg / kg) and other treatments. Control treatment showed the lowest amount of NH_4^+-N (56.5 mg/kg). In week 3, the highest amount of NH_4^+-N was found in soil treated with kariyat leaf extract (148.6 mg/kg), but this was not differ from those detected in the sample with tumaric rhizome extract (146.8 mg/kg) and some other treatments. Control treatment contained the lowest concentration of NH_4^+-N (56.8 mg/kg). Cinnamon bark treated soil had the highest amount of NH_4^+-N (166.9 mg/kg), which was not statistically different compared to those of bitter melon fruit treated soil (162.3 mg/kg) and some other treatments. Control treatment had the lowest amount of NH_4^+-N (67.1 mg/kg) in week 4.

3.3 Concentration of Nitrate Nitrogen (NO_3^--N) in the Soil

The concentrations of NO_3^- -N in the soil samples supplemented with Thai medicinal herb extracts significantly differed at weeks 1-3 (table 2). The sample with clove flower extract had the highest amount of NO_3^- -N (19.0 mg/kg). However such concentration was not different compared to that of the sample with cinnamon bark extract (15.4 mg/kg). Soil samples with extracts of ringworm bush leaf, pomegranate leaf, mangosteen fruit, thai copper pod leaf, Indian mulberry leaf, sweet basil leaf, lemon grass leaf, bitter cucumber fruit, egg woman stem, fingerroot stem, fingerroot rhizome and hog plum leaf did not contain NO_3^--N (0.0 mg/kg) in the first week. Soil sample with Siamese neem leaf extract showed the highest amount of NO_3^- -N (7.7 mg/kg), which did not differ from that found in the samples with pomegranate leaf extract (6.9 mg/kg) and control (5.5 mg/kg). Other treatments showed the lowest amount of NO_3^- -N (0.0 mg/kg) in week 2. Kariyat treated soil had the highest amount of NO_3^- -N (19.4 mg/kg) followed by control, guava leaf, sweet basil leaf and sugar apple leaf treated sample (6.9, 4.1, 4.1 and 1.3 mg/kg, respectively). Contrastly, other treatments showed the lowest amount of NO_3^- -N (0.0 mg/kg) in week 3. In week 4, the amount of NO_3^--N in the soils supplemented with Thai medicinal herb extracts did not differ significantly with the averages of NO_3^- -N ranging from 0.0 - 44.3 mg/kg.

3.4 The Percentage Ratios of NH_4^+-N: Inorganic N in the Soil

The percentage ratios of NH_4^+-N: inorganic N in the soil samples supplemented with Thai medicinal herb extracts showed a significant difference (table 3). Samples with the extracts of ringworm bush leaf, mangosteen fruit, Thai copper pod leaf, Indian mulberry leaf, sweet basil leaf, lemon grass leaf, bitter cucumber fruit, egg woman stem, fingerroot stem, fingerroot rhizome and hog plum leaf had the highest percentage ratio of NH_4^+-N: inorganic N (100 %). While clove flower incubated soil showed the lowest percentage ratio of NH_4^+-N: inorganic N (92.4 %) in the first week. In week 2, control treatment had the lowest percentage ratio of NH_4^+-N: inorganic N (90.5 %). Such percentage ratio, however, did not differ from those percentage ratios in the samples treated with pomegranate leaf extract (91.2 %) and Siamese neem leaf extract (93.6 %). Soil samples incubated with the extracts of ringworm bush leaf, heart-leaved moonseed stem, white pepper seed, pomegranate leaf, ginger rhizome, mangosteen fruit, Thai copper pod leaf, Siamese neem seed, Siamese neem leaf, banana fruit, clove flower, holy basil leaf, galanga rhizome, coffee ground, turmeric rhizome, bird's eye chilli fruit, Indian mulberry leaf, cinnamon bark, galanga stem, galanga leaf, bitter melon fruit, lemon grass leaf, bitter cucumber fruit, egg woman stem, fingerroot stem, fingerroot rhizome, kaffir lime leaf and hog plum leaf had the highest percentage ratio of NH_4^+-N: inorganic N (100 %), which was not significantly different compared to the percentage ratios found in soil samples mixed with extract of sugar apple leaf (99.1 %), guava leaf (96.7 %), and sweet basil leaf (96.5 %). In week 3, soil containing kariyat leaf extract had the lowest percentage ratio of NH_4^+-N: inorganic N (88.1 %). Soil with heart-leaved moonseed stem extract had the highest percentage ratio of NH_4^+-N: inorganic N (100 %), which did not differ from those of some other treatments. Soil with coffee ground extract had the lowest percentage ratio of NH_4^+-N: inorganic N (81.2 %) in week 4.

3.5 The Percentage Ratios of NO_3^- -N: Inorganic N in the Soil

The ratios of NO_3^- -N: inorganic N in the soil after incubation with Thai medicinal herb extracts were significantly different (table 3). The soil with clove flower extract had the highest ratio of NO_3^--N (7.5 %), which was not significantly different from those in soil samples with cinnamon bark (6.5 %), control (5.1 %), bird's eye chili fruit (4.4 %), and sugar apple leaf (4.2 %). Samples with the extracts of ringworm bush leaf, pomegranate leaf, mangosteen fruit, Thai copper pod leaf, Indian mulberry leaf, sweet basil leaf, lemon grass leaf, bitter cucumber fruit, egg woman stem, fingerroot stem, fingerroot rhizome and hog plum leaf showed the lowest ratio of NO_3^--N (0.0 %) in the first week. Control had the highest ratio of NO_3^--N (9.4 %), which was not significantly different from the ratios in samples with extracts of pomegranate leaf (8.71 %) and Siamese neem leaf (6.4 %) .

Other treatments showed the lowest ratio of NO_3^--N (0.0 %) in week 2. Soil treated with kariyat leaf extract had the highest ratio of NO_3^{-}N (11.8 %) %), which was not significantly different compared to that of control (11.3 %). Other treatments showed the lowest ratio of NO_3^--N (0.0 %) in week 3. Coffee ground treated sample had the highest ratio of NO_3^--N (18.7 %), which did not differ from some other treatments. Only the heart-leaved moonseed stem treated sample showed the lowest ratio of NO_3^--N (0.0 %).

Table 2. Concentrations of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) in the soil supplemented with Thai medicinal herb extracts during incubation

Medicinal Herbs	**NH_4^+-N (mg/kg)**				**NO_3^- -N (mg/kg)**			
	week 1	week 2	week 3	week 4	week 1	week 2	week 3	week 4
Control	134.1 f	56.5 e	56.8 f	67.2 d	7.3 bcd	5.5 a	7.0 b	8.9
Banana fruit	197.9 abc	116.7 abc	132.2 a-d	139.9 abc	0.9 f	0.0 b	0.0 c	4.0
Bird's eye Chilli fruit	165.4 c-f	80.4 cde	123.6 a-d	144.5 abc	8.2 bc	0.0 b	0.0 c	9.7
Bitter Cucumber fruit	144.4 ef	08.3 a-d	119.4 a-e	147.9 abc	0.0 f	0.0 b	0.0 c	15.4
Bitter Melon fruit	174.4 c-f	102.6 a-d	125.1 a-d	162.4 a-b	3.6 c-f	0.0 b	0.0 c	13.7
Cinnamon bark	219.9 ab	122.1 ab	137.3 a-d	167.0 a	15.5 a	0.0 b	0.0 c	10.5
Clove flower	233.4 a	131.4 a	140.1 abc	143.6 abc	19.2 a	0.0 b	0.0 c	12.9
Coffee ground	183.6 b-e	84.4 cde	113.9 b-e	133.1 abc	4.5 b-f	0.0 b	0.0 c	44.3
Egg Woman stem	158.3 c-f	100.9 a-d	123.7 a-d	146.2 abc	0.0 f	0.0 b	0.0 c	14.5
Fingerroot rhizome	185.1 b-e	103.6 a-d	106.5 de	136.7 abc	0.0 f	0.0 b	0.0 c	12.9
Fingerroot stem	172.4 c-f	102.7 a-d	109.5 cde	135.8 abc	0.0 f	0.0 b	0.0 c	17.0
Galanga leaf	197.1 abc	94.3 a-d	122.4 a-d	141.5 abc	3.6 c-f	0.0 b	0.0 c	18.6
Galanga rhizome	179.4 b-e	80.5 b-e	116.4 a-e	131.6 abc	3.6 c-f	0.0 b	0.0 c	9.7
Galanga stem	190.8 bcd	94.2 a-d	113.9 b-e	146.2 abc	1.8 ef	0.0 b	0.0 c	10.5
Ginger rhizome	165.8 c-f	102.3 a-d	124.7 a-d	140.8 abc	0.9 f	0.0 b	0.0 c	7.3
Guava leaf	189.1 b-d	117.9 abc	127.8 a-d	143.8 abc	6.4 b-e	0.0 b	4.2 bc	8.9
Heart-leaved moonseed stem	161.9 c-f	102.5 a-d	126.0 a-d	130.3 bc	0.9 f	0.0 b	0.0 c	0.0
Hog Plum leaf	167.8 c-f	111.2 a-d	126.1 a-d	151.7 abc	0.0 f	0.0 b	0.0 c	16.1
Holy basil leaf	163.8 c-f	84.5 b-e	107.0 cde	128.1 bc	0.9 f	0.0 b	0.0 c	10.5
Indian Mulberry leaf	164.1 c-f	95.7 a-d	121.9 a-d	154.8 abc	0.0 f	0.0 b	0.0 c	13.7
Kaffir lime leaf	158.0 c-f	110.8 a-d	117.9 a-e	146.4 abc	1.8 ef	0.0 b	0.0 c	15.4
Kariyat leaf	180.5 b-e	110.7 a-d	148.6 a	139.0 abc	4.5 b-f	0.0 b	19.4 a	8.9
Lemon Grass leaf	171.6 c-f	115.2a-d	121.9 a-d	157.0 abc	0.00 f	0.0 b	0.0 c	17.8
Mangosteen fruit	169.8 c-f	103.9 a-d	130.4 a-d	140.5 abc	0.00 f	0.0 b	0.0 c	4.8
Pomegranate leaf	148.5 d-f	84.6 b-e	117.8 a-e	127.5 bc	0.00 f	7.0 a	0.0 c	15.3
Ringworm Bush leaf	149.8 d-f	99.6 a-d	112.4 cde	149.8 abc	0.00 f	0.0 b	0.0 c	3.6
Siamese neem leaf	179.5 b-e	104.8 a-d	128.1 a-d	127.7 bc	0.9 f	7.8 a	0.0 c	14.6
Siamese neem seed	188.0 b-e	77.9 de	88.8 e	83.7 d	7.3 bcd	0.0 b	0.0 c	13.7
Sugar Apple leaf	190.0 bcd	94.3 a-d	120.6 a-e	131.7 abc	9.1 b	0.0 b	1.4 c	11.3
Sweet basil leaf	161.8 c-f	105.3 a-d	128.3 a-d	147.9 abc	0.0 f	0.0 b	4.1 b	12.1
Thai copper pod leaf	164.2 c-f	107.1 a-d	123.8 a-d	136.4 abc	0.0 f	0.0 b	0.0 c	5.7
Turmaric rhizome	161.8 c-f	112.4 a-d	146.9 ab	143.4 abc	4.5 b-f	0.0 b	0.0 c	11.3
White pepper seed	175.3 c-f	90.1 b-e	111.0 cde	125.1 c	2.7 def	0.0 b	0.0 c	5.6
p-value	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
CV (%)	15.1	20.7	17.1	16.8	12.7	22.4	19.5	10.6

Note. Mean values in each column followed by the same letter are not significantly different by DMRT at P≤ .05.

Table 3. The percentage ratios of NH_4^+-N: inorganic N and NO_3^--N: inorganic N in the soil supplemented with Thai medicinal herb extracts during incubation

[redacted]

Note. Mean values in each column followed by the same letter are not significantly different by DMRT at P≤ .05.

3.6 pH Values of the Soil

The pH values of the soil samples treated with various Thai medicinal herb extracts differed significantly (table 4) over the incubation period. Sample treated with clove flower extract had the highest pH (5.6) at week 1. Such pH, however, was not different from those of the sample treated with cinnamon bark extract (5.5) and control (5.2). The sample treated with bird's eye chilli extract had the lowest pH (3.5). Control had the highest pH (5.6) in week 2 and soil sample with hog plum extract had the lowest pH (3.8). In week 3, control had the highest pH (5.3), which was not different compared to those of the samples with extracts of clove flower, bitter cucumber fruit, ringworm bush leaf, Thai copper pod leaf, Siamese neem seed, Siamese neem leaf, holy basil leaf, tumaric rhizome, sweet basil leaf, lemon grass leaf, egg woman stem, kaffir lime leaf and hog plum leaf. In week 3, coffee ground treated sample had the lowest pH value (4.5), which was not significantly different from those of the samples treated with the extracts of ginger rhizome (4.6), galanga rhizome (4.6) and sugar apple leaf (4.6). In week 4, Siamese neem seed had the highest pH value (5.5), which did not significantly differ from those of the samples containing extracts of Thai copper pod leaf, kariyat leaf, guava leaf and clove flower. Samples treated with extracts of holy basil leaf, Indian mulberry leaf and bitter melon fruit had the lowest pH value (4.7). pH (5.6) in week 2 and soil sample with hog plum extract had the lowest pH (3.8). In week 3, control had the highest pH (5.3), which was not different compared to those of the samples with extracts of clove flower, bitter cucumber fruit, ringworm bush leaf, Thai copper pod leaf, Siamese neem seed, Siamese neem leaf, holy basil leaf, tumaric rhizome, sweet basil leaf, lemon grass leaf, egg woman stem, kaffir lime leaf and hog plum leaf. In week 3, coffee ground treated sample had the lowest pH value (4.5), which was not significantly different from those of the samples treated with the extracts of ginger rhizome (4.6), galanga rhizome (4.6) and sugar apple leaf (4.6). In week 4, Siamese neem seed had the highest pH value (5.5), which did not significantly differ from those of the samples containing extracts of Thai copper pod leaf, kariyat leaf, guava leaf and clove flower. Samples treated with extracts of holy basil leaf, Indian mulberry leaf and bitter melon fruit had the lowest pH value (4.7).

Table 4. pH values of the soil treated with Thai medicinal herb extracts during 4 weeks incubation

Medicinal Herbs	**pH (1:1)**				**Medicinal Herbs**	**pH (1:1)**			
	week 1	week 2	week 3	week 4		week 1	week 2	week 3	week 4
Control	5.2 ab	5.6 a	5.4 a	5.1 b-g	Holy basil leaf	3.7 fg	3.9 gh	5.2 abc	4.8 h
Banana fruit	4.1 c-f	4.5 b-g	4.8 def	5.3 bcd	Indian Mulberry leaf	3.7 fg	4.3 e-h	4.9 c-f	4.8 h
Bird's eye Chilli fruit	3.5 g	4.1 e-h	4.9 c-f	5.1 b-g	Kaffir lime leaf	3.9 c-g	4.1 e-h	5.2 abc	4.9 e-h
Bitter Cucumber fruit	3.6 fg	4.2 e-h	5.4 a	5.2 b-g	Kariyat leaf	3.7 efg	4.3 d-h	5.0 b-f	5.3 ab
Bitter Melon fruit	3.7 efg	4.2 e-h	4.7 ef	4.8 h	Lemon Grass leaf	4.0 c-g	4.4 c-h	5.0 a-e	5.2 b-f
Cinnamon bark	5.5 a	4.9 bcd	4.8 def	5.1 b-g	Mangosteen fruit	4.1 c-f	4.5 b-g	4.9 c-f	5.0 c-h
Clove flower	5.6 a	4.9 bc	5.4 a	5.3 abc	Pomegranate leaf	3.7 fg	4.4 c-h	4.9 def	5.0 d-h
Coffee ground	3.9 c-g	3.9 gh	4.6 f	5.0 d-h	Ringworm Bush leaf	3.8 d-g	4.5 b-g	5.1 a-e	5.1 b-g
Egg Woman stem	3.7 fg	4.4 b-h	5.2 abc	5.0 e-h	Siamese neem leaf	3.8 efg	4.4 c-h	5.1 a-e	4.9 e-h
Fingerroot rhizome	4.3 cde	4.3 e-h	4.8 def	5.2 b-f	Siamese neem seed	5.0 b	4.7 b-e	5.2 abc	5.6 a
Fingerroot stem	3.8 efg	4.2 e-h	4.7 e-f	5.2 b-g	Sugar Apple leaf	3.9 d-g	4.2 e-h	4.6 f	5.0 d-h
Galanga leaf	3.7 efg	5.0 b	4.9 c-f	5.2 b-f	Sweet basil leaf	4.3 cd	4.5 b-g	5.0 a-e	4.9 fgh
Galanga rhizome	3.8 d-g	4.0 fgh	4.6 f	4.9 gh	Thai copper pod leaf	3.8 d-g	4.6 b-g	5.1 a-e	5.4 ab
Galanga stem	3.9 c-g	4.2 e-h	4.9 b-f	5.2 b-g	Turmaric rhizome	3.8 d-g	4.6 b-f	5.1 a-d	5.0 c-h
Ginger rhizome	4.4 c	4.6 b-f	4.6 f	5.2 b-e	White pepper seed	4.0 c-g	4.2 e-h	4.9 c-f	5.3 bcd
Guava leaf	3.7 efg	4.3 e-h	4.9 b-f	5.4 ab	*p-value*	0.0	0.0	0.0	0.0
Heart-leaved moonseed stem	4.0 c-g	4.4 c-h	4.9 b-f	5.0 c-h	CV (%)	14.5	10.6	5.9	4.8
Hog Plum leaf	3.8 d-g	3.8 h	5.7 ab	5.2 b-f					

Note. Mean values in each column followed by the same letter are not significantly different by DMRT at P ≤ .05.

4. Discussion

4.1 Effect of Thai Medicinal Herb Extracts on the Population of AOB and NOB

The plant extracts decreased the numbers of nitrifying bacteria, both AOB and NOB. The extract of galanga stem greatly decreased the numbers of AOB at 1 and 2 weeks of incubation. Holy basil leaf and lemon grass leaf extracts decreased the numbers of AOB during the first week only. Bitter melon fruit extract reduced the numbers of AOB down in week 2. The AOB numbers were lower in samples treated with the extract of sugar apple leaf, egg woman stem, fingerroot stem, fingerroot rhizome and kaffir lime leaf during week 3. The extracts of bird's eye chilli fruit and galanga stem were able to decrease the numbers of NOB well during the first 2 weeks, while those of ginger rhizome, Siamese neem leaf, guava leaf and banana fruit decreased the numbers of

NOB in the soil during weeks 2-3. It was observed that the extracts of ringworm bush leaf, Thai copper pod leaf, heart-leaved moonseed stem, mangosteen fruit, kariyat leaf and galanga rhizome could greatly lower the numbers of NOB in the soil after the first week. It could be clearly observed that the plant extracts decreased the numbers of AOB only during the first 3 weeks, and decreased the numbers of NOB after the first week of incubation. This was probably because herb extracts contained substances that could inhibit nitrification and microbial activity of nitrifying bacteria. In studies on biological nitrification inhibition (BNI) activity of *Brachiaria humidicola*, Ishikawa et al. (2003) detected a decrease in AOB population size in an Andosol soil, whereas Gopalakrishnan et al. (2009) found no differences in AOB counts. Which give the same effect on the use of chemicals such as study of Hua et al.(2008) showed that DMPP decreased AOB population, whereas, it did not decrease NOB population. But this study found that herb extracts decreased the numbers of NOB after the first week of incubation. The growth of NOB was observed to be inhibited in the presence of high ammonia levels (Kim et al., 2005). High ammonium contentration in soil decreased the growth and activity of nitrifying bacteria in *Nitrobacter* sp. (Prosser, 2007).

4.2 Effect of Thai Medicinal Herb Extracts on the Concentration of Ammonium Nitrogen (NH_4^+-N) and Nitrate Nitrogen (NO_3^--N)

It was found that the concentrations of nitrogen in NH_4^+-N form found in all samples treated with herbal extracts were higher than that of control throughout the incubation period. In similar study, Wu et al., (2007) showed that DMPP-amended soil led to the maintenance of relatively high levels of NH_4^+-N and low levels of NO_3^--N in soil, and nitrification was slower. It can be seen that the use of herb extracts, the result was similar to the use of chemicals such as DMPP. The amount of NH_4^+-N was high in the first week and greatly reduced in weeks 2 before slightly increased in weeks 3 and 4. This was probably because herb extracts contained substances that could inhibit nitrification and microbial activity of nitrifying bacteria. The conversion of ammonium nitrogen to nitrate nitrogen is slow. As a result, the amount of nitrate nitrogen decreased by inhibiting oxidation of ammonium to nitrite and ammonium can remain in the soil longer. The results were consistent to the study of Haile *et al*. (2006), reported that herbal extracts influenced the amount of NH_4^+-N as well as NO_3^- -N reduction, therefore, nitrification was inhibited. The concentrations of nitrogen in NO_3^--N form in soil samples with herbal extracts were lower than that of control throughout the incubation period. Soil samples with extracts of ringworm bush leaf, mangosteen fruit, Thai copper pod leaf, Indian mulberry leaf, lemon grass leaf, bitter cucumber fruit, egg woman stem, fingerroot stem, fingerroot rhizome and hog plum leaf had the lowest amount of NO_3^- -N during the first 3 weeks. The soil supplemented with heart-leaved moonseed stem extract contained the lowest amount of NO_3^- -N after weeks 2 until the end of the incubation period in weeks 4. Other treatments, however, showed the lowest amount of NO_3^- -N during weeks 2 and 3 only. The herb extract provide results similar to use of chemicals. Hua et al. (2008), Iqbal et al. (2012) and Yu et al. (2007) showed that the nitrification inhibitor thiourea inhibited the nitrate loss in the leaching samples and the concentration of nitrate became low as by inhibiting the nitrification loss.

4.3 Influence of Thai Medicinal Herb Extracts on the Percentage Ratios of NH_4^+-N: Inorganic N and NO_3^- -N: Inorganic N in the Soil

The higher percentage ratios of NH_4^+-N: inorganic N in soil indicated higher effectiveness of nitrification inhibition since the NH_4^+-N to NO_3^--N conversion was slower than that of control. The ratios, which is considered a better and more sensitive indicator of effectiveness nitrification inhibitor (Hauck, 1984). If the percentage ratios of NH_4^+-N is very high, it indicates that the substances can inhibit nitrification as well. Which nitrification inhibitors reduce emission of N_2O, directly by reducing nitrification, and indirectly by reducing the availability of NO_3^- for denitrification (McTaggart et al., 1997; Castaldi & Smith, 1998; Majumdar et al., 2000; Pathak & Nedwell, 2001). The herb extract provide results similar to use of chemicals. The nitrification inhibitor DMPP (Hua et al., 2008; Iqbal et al.,2012; Yu et al.,2007) or thiourea (Wu et al., 2007) inhibited the nitrate loss in soil and high levels of NH_4^+-N by inhibiting the nitrification loss. This indicates that herb extracts was effective nitrification inhibitor. Extracts of ringworm bush leaf, mangosteen fruit, Thai copper pod leaf, Indian mulberry leaf, lemon grass leaf, bitter cucumber fruit, egg woman stem, fingerroot stem, fingerroot rhizome, hog plum leaf and heart-leaved moonseed stem inhibited nitrification in soil for three weeks. Other treatments had an effect on nitrification inhibition in soil for just two weeks. Control and soil samples treated with extracts of ground coffee, kariyat leaf and cinnamon bark had higher ratios of NO_3^- -N: inorganic N than any other herb extracts. This could be because those samples contained none or very small amount of nitrification inhibitors, therefore the conversion from NH_4^+-N to NO_3^- -N occurred quickly. Sample treated with other herb extracts had low ratios of NO_3^- -N. This could be due to the presence of some substances that could inhibit nitrification. The effects of those extracts were found to be low in early stages of incubation. This may be because the active substances were slowely released. The inhibition effects was more pronounce, especially in weeks 2 and 3. This probably because the inhibiors were released more during this stages. High inhibition effects were observed in samples treated with extracts of ringworm bush leaf, mangosteen fruit, Thai copper pod leaf, Indian mulberry leaf, lemon grass leaf, bitter cucumber fruit, egg woman stem, fingerroot stem, fingerroot rhizome, hog plum leaf and heart-leaved moonseed stem. This was probably due to the active ingredients in those extracts, which were able to inhibit nitrification. The results showed those extracts released nitrification inhibitors for up to 3 weeks. After that, the inhibition effects decreased.

4.4 Effect of Thai Medicinal Herb Extracts on Soil pH

It was observed that in week 1, the pH values of the samples with Thai medicinal herb extracts were generally lower than that of control. In similar study, Kyveryga et al. (2004) showed that 89 % nitrification of fertilizer N in soils having pH > 7.5 and 39 % nitrification of this N in soils having pH < 6.0. It is well established, however, that nitrification is relatively slow at pH values < 5. (Alexander, 1965; Sahrawat, 1982; Schmidt, 1982; Ruiz et al., 2003). This could be because the release of the active ingredients from the herbal extracts was fast, therefore nitrification inhibition effects were high. The conversion of NH_4^+-N to NO_3^- -N occurred rapidly in the first week, then the release of H^+ was high. The active ingredients from the herbal extracts was released later on, and brought the pH values up, which could be observed after 3 weeks. It can be seen from the results that the pH of the samples treated with herbal extracts increased over time.

5. Conclusion

Addition of some Thai medicinal herb extracts to the soil resulted in higher concentration of NH_4^+-N, lower concentration of NO_3^--N and higher NH_4^+-N: inorganic N ratio compared to those in control. Soil treated with extracts of ringworm bush leaf, mangosteen fruit, Thai copper pod leaf, Indian mulberry leaf, lemon grass leaf, bitter cucumber fruit, egg woman stem, fingerroot stem, fingerroot rhizome, hog plum leaf and heart-leaved moonseed stem had a ratio of NH_4^+-N: inorganic N of 100% for a longer period up to 3 weeks. The pH of treated soils were rather low at early stages of incubation, best gradually increased over time. The herbal extracts could significantly decrease the numbers of AOB and NOB. Galanga stem extract had more efficiency on decreasing the numbers of AOB then the others. Extracts of ringworm bush leaf, Thai copper pod leaf, heart- leaved moonseed leaf, mangosteen fruit, kariyat leaf and galangal rhizome had more efficiency on decreasing the numbers of NOB then the others after first week. These medicinal herbs had a high potential for addition with nitrogen fertilizer in order to protect the nitrogen loss from soil. Anyhow, the appropriate quality of medicinal herbs should be determined in the field before recommendation.

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