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Inhibitory Effect of Neem Seed Extract on Soil Nitrogen Mineralization under Chemical and Organic Amendments

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ABSTRACT

Background: Improvement of nitrogen use efficiency (NUE) of chemical fertilizers and N-rich-organic amendments using natural mineralization inhibitors, like neem extract, was recommended to minimize nitrogen (N) loss. This study hence aimed to evaluate the effects of combined uses of neem seed extract with cricket feces on soil N mineralization and plant NUE.

Methods: The treatment combinations included i) amending materials involving unamended (Un), chemical fertilizer at a recommended rate of 312.5 kg N ha^{-1}, 100 kg P ha^{-1} and 100 kg K ha^{-1} (CF) and three rates of cricket feces (CrF) [3.125 (CrF_{low}), 6.25 (CrF_{medium}) and 12.5 (CrF_{high}) Mg ha^{-1}], ii) in combination without (-Nm) and with (+Nm) neem seed extract. Amaranth (*Amaranthus tricolor*) was employed to assay the plant's NUE.

Result: Neem seed extract inhibited the N mineralization under the Un (74%) and CF (84%) treatments, but not in any of the CrF treatments (-102%, -99% and -2% for CrF_{low}, CrF_{medium} and CrF_{high}, respectively. In addition, the neem extract significantly decreased NUE parameters, including N recovery efficiency and agronomy NUE, only in CF treatment. Neem seed extract could only function as an N inhibitor in unamended and chemically fertilized soils.

Key words: Mineralization inhibition, Nitrogen use efficiency, Nitrogen-rich material, Waste recycling.

INTRODUCTION

Nitrogen (N) loss from cultivated land through the N transformation process causes many environmental pollutions, *e.g.*, nitrate contaminations of surface- and groundwater and nitrous oxide emission (Benckiser *et al.*, 2015). These pollutions consequently affect severe human health risks. The environmental contaminations of N are attributed both to chemical fertilizers and N-rich-organic amendments (Bijay and Craswell, 2021). Enhancing N use efficiency (NUE) by applying an N-release controller such as N mineralization inhibitors has been recommended to minimize environmental hazards due to N leaking (Singh *et al.*, 2019). Economically, this strategy can save chemical fertilizer costs under its expensive crisis nowadays.

Many chemicals are employed as soil N mineralization inhibitors; nevertheless, they are costly and unavailable in traditional markets (Kumar *et al.*, 2010). Neem extract has been reported as a natural N mineralization inhibitor and improves NUE of plant (Meena *et al.*, 2021).

Several organic amendments were recently promoted for soil fertility improvement, substituting chemical fertilizers to address the negative environmental impact of industrial fertilizer production and ease the expensive cost crisis (Galic *et al.*, 2020). Cricket-keeping farms have continuously grown up in many countries in Asia and Africa (Halloran *et al.*, 2017). In Thailand alone, over 20,000 farms were observed, where released cricket feces at an average of 44 Mg $farm^{-1}$ $year^{-1}$ (Halloran *et al.*, 2017). Recycling the feces into organic amendment can create economic value and simultaneously decrease environmental problems. However, a very high N content, 2.3-2.6% N, of cricket feces (Halloran *et al.*, 2017) may be mineralized and lost rapidly. Application of inhibition inhibitor may be a solution to the aforementioned issues. Yet, to our knowledge, neem extract application as an N mineralization inhibitor in soil amended with N-rich organic material has not been reported.

The current paper hypothesized that combining cricket feces with neem seed extract would inhibit soil N mineralization and improve the NUE of the plant. Therefore, the objective of this paper aimed to estimate the effects of the combination uses of cricket feces with neem seed extract on soil N mineralization and plant's NUE.

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MATERIALS AND METHODS

Soil, cricket feces and neem seed extract

The Roi-et soil series (isohyperthermic Aeric Kandiaquults) was collected from a research field in Sakon Nakhon

Rajabhat University, Sakon Nakhon, Thailand, at a depth of 1-15 cm. Cricket (*Acheta domesticus*) feces was obtained from a farm located in Sakon Nakhon. The soil and cricket feces were air-dried and sieved through 2-mm mesh. The beginning characteristics of soil and cricket feces are shown in Table 1.

Neem (*Azadirachta indica*) seed extract was a commercially available agricultural product made from neem seed cake by a mill factory in Nakhon Ratchasima, Thailand. The extract contained 1375 mg N L^{-1} and 12.6 mg azadirachtin mL^{-1}.

Pot trail

A pot trial was conducted under greenhouse from January to February 2020. A factorial treatment structure was employed with five soil amendments: unamended (Un); chemical fertilizer (CF) and three rates of cricket feces: 3.125 Mg ha^{-1} (CrF_{low}), 6.25 Mg ha^{-1} (CrF_{medium}) and 12.5 Mg ha^{-1} (CrF_{high}). These amendments were combined with two rates of neem seed extract: without (-Nm) and with (+Nm) extract. A total of ten treatment combinations were arranged in a completely randomized design (CRD) with three replications. Amaranth (*Amaranthus tricolor*) was used to evaluate NUE.

Two kilograms of air-dry soil were added to a pot (d=15 cm, h=14 cm and v=2085 cm^3). Cricket feces rates were accordingly added, mixed thoroughly and incubated in the soil for 15 days before amaranth transplanting. Meanwhile, a recommendation rate of chemical fertilizer (312.5 kg N ha^{-1}, 100 kg P ha^{-1} and 100 kg K ha^{-1}) regarding Chakhatrakan (2003) was applied equally two times to the CF treatments at 18 and 30 days after planting. A commercial amaranth variety was planted and nursed for 15 days and a single seedling was transplanted to each pot. Fifty milliliters of neem extract at a concentration of 0.1 mg azadirachtin ml^{-1} was added to each +Nm pot once a week at 15, 22, 29 and 36 days after planting. The rate of neem extract was modified according to Sarawaneeyaruk *et al.* (2015). The soil moisture content of each pot was maintained to 65% of water holding capacity throughout the pot trail. At 39 days after planting, amaranth was cut to evaluate the NUE. Fresh soil was sampled on the same day for mineral N determination.

Laboratory analyses

Soil particle size distribution was determined by the pipette method (Kroetsch and Wang, 2008). The bulk density of soil and cricket feces was measured using the core method (Pansu and Gautheyrou, 2006). Electrical conductivity and pH were determined using the soil-to-water ratio of 1:5. Organic carbon determination was achieved by the Walkley and Black method (Nelson and Sommers, 1982). Determination of cation exchange capacity was performed following Pansu and Gautheyrou (2006).

Mineral N was extracted in 2 *M* KCl solution (Stevenson, 1982) and determined using the distillation method on a micro-Kjeldahl distillation (Pro-Nitro S 4002851, JP Selecta, Barcelona). Microbial activity was determined according to the fluorescein diacetate hydrolysis method described by Green *et al.* (2006).

Data calculations

The calculation of the net N mineralization rate (Net N min rate) was modified from Bi *et al.* (2017):

$$\text{Net N min rate (mg N kg}^{-1}\text{ soil day}^{-1}) = \frac{[\text{Mineral N}]_{t2} - [\text{Mineral N}]_{t1}}{t}$$

Where,

$[\text{Mineral N}]_{t2}$ and $[\text{Mineral N}]_{t1}$ are soil mineral N concentrations at the harvest and the start of the experiment, respectively.

The N mineralization inhibition was computed using an equation modified from Aspelin and Ekholm (2017) as follows:

$$\text{N mineralization inhibition (\%)} = \frac{(\text{Net N min rate})_{un} - (\text{Net N min rate})_{am}}{(\text{Net N min rate})_{un}}$$

Where,

$(\text{Net N min rate})_{un}$= Net N mineralization rate of Un treatments.

$(\text{Net N min rate})_{am}$= Treatments of CF, CrF_{low}, CrF_{medium} and CrF_{high}.

Nitrogen recovery efficiency (NRC) and agronomic nitrogen use efficiency (AgrNUE) were calculated by modifying the method of Carranca (2012) through the following equations:

$$\text{NRC (\%)} = \frac{(\text{N uptake})_{am} - (\text{N uptake})_{un}}{\text{N input}} \times 100$$

Where,

$(\text{N uptake})_{am}$ = N uptake by amaranth under CF, CrF_{low}, CrF_{medium} and CrF_{high} treatments.

$(\text{N uptake})_{un}$ = N uptake under Un treatments and N input is an amount of added N.

$$\text{AgrNUE (g DW g}^{-1}\text{ input)} = \frac{(\text{Shoot DW})_{am} - (\text{Shoot DW})_{un}}{\text{N input}}$$

Where,

$(\text{Shoot DW})_{am}$ = Shoot dry weight of amaranth under the CF, CrF_{low}, CrF_{medium} and CrF_{high} treatments.

$(\text{Shoot DW})_{un}$ = Shoot dry weight under Un treatments.

N input = An amount of added N.

Statistical analysis

A two-way analysis of variance based on a factorial arrangement in CRD was used to estimate the effects of combined uses of neem seed extract with soil amendments on soil N transformation and N use efficiencies of the amaranth. Treatment mean comparisons were carried out *via* the Tukey's honest significant difference "(HSD)" test. The principal component analysis (PCA) using the PROC PRINCOMP model was performed to identify the relationships among parameters of soil N status and N use efficiency. Statistical analyses were done following SAS Institute Inc (2004). Significant differences were at $P \leq 0.05$.

RESULTS AND DISCUSSION

Inhibitory effect of neem seed extract on N mineralization occurring only in soils without cricket feces amendment

Soil mineral N concentrations and net N mineralization rates under Un and CF treatments significantly decreased in +Nm treatments relative to -Nm (Table 2). However, the opposite results were found in all cricket feces treatments. Soil mineral N concentrations and net N mineralization rates in -Nm and +Nm increased significantly with greater rates of cricket feces. Meanwhile, N mineralization inhibition occurred in Un and CF treatments under +Nm, but *vice versa* in the cricket feces treatments.

The inhibitory effect on N mineralization under Un+Nm and CF+Nm was attributed to the neem active ingredients: azadirachtin, salanin, 14-epoxiazadiradione, meliantrol, melianone, gedunin, nimboline, nimbin, deacetilasalanin, azadiractol, azadirone, vilosinin and meliacarpine (Choudhury *et al.*, 2016). These ingredients have been reported to inhibit the N mineralization sub-processes, *i.e.*, urea hydrolysis (Mohanty *et al.*, 2008) and nitrification (Alves *et al.*, 2009). Among these ingredients, azadirachtin was reported to serve as an essential mineralization inhibitor (Sarawaneeyaruk, Krajangsang and Pringsulaka, 2015). The current study's azadirachtin content of neem seed extract was 12.6 mg mL^{-1}.

Instead of inhibition, combinations of neem seed extract with cricket feces stimulated N mineralization, as elucidated in the negative values of N mineralization inhibition (Table 2). The denature of neem ingredients might be a consequence of malfunction of the inhibitory property. Sundaram *et al.* (1995) reported that azadirachtin was denatured via hydrolytic and microbial degradations. The higher degree of azadirachtin hydrolytic degradation occurred in the higher pH values in the following orders: pH 10 >>> pH 7 > pH 4. In the current study, pH values of soils under +Nm combined with CrF_{low}, CrF_{medium} and CrF_{high} were 5.90, 5.93 and 6.29, respectively (Table 2). Meanwhile, pHs of Un+Nm and CF+Nm were 5.90 and 5.82, respectively. Therefore, lower pH of the unamended and chemical fertilizer treatments than cricket feces amended soils (CrF_{medium} and CrF_{high}) implied that less hydrolysis degradation of azadirachtin existed in the former soils.

Another malfunction of inhibitory property of neem seed extract in the cricket feces amended soils might be a consequence of microbial degradation of azadirachtin. Microorganisms were reported to play a crucial role in decomposing azadirachtin (Stark and Walter, 1995). The current study showed significantly higher microbial activity, indicated by fluorescein released, in cricket feces amended treatments than the Un and CF treatments (Table 2). In addition, the higher rates of cricket feces brought about higher degrees of microbial activity, as shown by significantly

Table 1: Initial properties of soil and cricket feces.

Material	Soil particle distribution (%)			Soil texture	BD †	pH	EC (1:5)	Organic C	CEC	Mineral N
	Sand	Silt	Clay		(g cm^{-3})	(1:5)	(mS cm^{-1})	(g kg^{-1})	(cmol kg^{-1})	(mg kg^{-1})
Soil	78.5	15.7	5.8	Loamy sand	1.48	5.97	0.042	3.83	3.63	4.12
Cricket feces	-	-	-	-	0.32	8.39	15.79	332	68.4	1488

† BD= Bulk density; EC= Electrical conductivity; CEC= Cation exchange capacity.

Table 2: Soil pH, fluorescein released and N status as affected by the combined uses of cricket feces and neem seed extract.

Amendment †	pH (1:5) (mg kg^{-1})	Fluorescein released (mg kg^{-1})	Mineral N	Net N mineralization rate (mg N kg^{-1} soil day^{-1})	N mineralization inhibition (%)
Un - Nm	5.90 c ‡	134 d	4.12 c	0.076 c	-
CF - Nm	5.82 d	132 d	3.46 d	0.059 d	-
CrF_{low} - Nm	5.90 c	143 c	2.70 e	0.039 e	-
CrF_{medium} - Nm	5.98 b	145 c	3.39 d	0.057 d	-
CrF_{high} - Nm	6.29 a	172 a	4.36 c	0.082 c	-
Un + Nm	5.90 c	146 c	1.94 f	0.020 f	+73.8 a
CF + Nm	5.82 d	135 d	1.54 f	0.010 f	+83.7 a
CrF_{low} + Nm	5.90 c	141 c	4.26 c	0.079 c	-101.8 c
CrF_{medium} + Nm	5.93 bc	146 c	5.56 b	0.113 b	-97.8 c
CrF_{high} + Nm	6.29 a	164 b	6.10 a	0.126 a	-2.0 b
P-value	<0.001	<0.001	<0.001	<0.001	<0.001
F-test	***	***	***	***	***
CV (%)	0.42	2.51	7.17	10.43	334.5

*** Significant at P≤0.001.

† Un= Unamended; CF= Chemical fertilizer; CrF_{low}, CrF_{medium} and CrF_{high}= Cricket feces at rates of 3.125, 6.25 and 12.5 Mg ha^{-1}, respectively and -Nm and +Nm= Without and with neem seed extract.

‡ Means within the same column followed by the same letter are not significantly different at P≤0.05 (Tukey's HSD test).

higher fluorescein released in CrF_{high} than CrF_{low} and CrF_{medium}. Cricket feces was a vital source of energy and nutrients for soil microorganisms, as evidenced by the high contents of organic C and mineral N (Table 1). Our results agreed with Agyarko *et al.* (2006), who determined that higher degrees of azadirachtin degradation were rendered by increased amounts of poultry and cattle manures.

A significant increase in N mineralization inhibition under CrF_{high} relative to its lower cricket feces rates (Table 2) might be manifested by the decomposing products derived from the feces, such as humic substances and organic molecules. Humic acid was reported as a nitrification inhibiting compound (Benckiser, Schartel and Weiske, 2015). In addition, several organic molecules derived from the decomposition, *e.g.*, quinines, catechols, ethylene, acetylene, gallic acid and tartaric acid, were reported to bring about microbial toxicity (Kaal *et al.*, 2012). Even though organic molecule concentration was not determined in this paper, higher rates of cricket feces were assumed to increase the content of soil organic molecules. This assertion was in line with Nair *et al.* (2015) who stated that the greater input of farm yard manure brought about higher soil organic content.

Inhibition of N mineralization diminishing nitrogen use efficiencies of a vegetable amaranth

Significant decreases in N use efficiencies, involving NRE and AgrNUE, under CF+Nm compared to CF-Nm (Table 3) resulted from N mineralization inhibition. This effect was

Table 3: Nitrogen (N) uptake and N use efficiencies [involving N recovery efficiency (NRE) and Agronomic N use efficiency (AgrNUE)] of amaranth as affected by the combined uses of cricket feces and neem seed extract.

Amendment †	N uptake (mg plant^{-1})	N use efficiency	
		NRE (%)	AgrNUE (g DW g^{-1} N input)
Un - Nm	0.34 ef ‡	-	-
CF - Nm	1.03 d	0.78 a	20.3 a
CrF_{low} - Nm	1.78 b	0.74 ab	18.9 a
CrF_{medium} - Nm	2.71 a	0.61 bc	14.6 b
CrF_{high} - Nm	1.65 bc	0.17 d	4.2 c
Un + Nm	0.20 f	-	-
CF + Nm	0.80 de	0.51 c	14.5 b
CrF_{low} + Nm	1.97 b	0.84 a	20.0 a
CrF_{medium} + Nm	2.65 a	0.59 bc	14.6 b
CrF_{high} + Nm	1.24 cd	0.12 d	2.6 c
P-value	<0.001	<0.001	<0.001
F-test	***	***	***
CV (%)	12.35	10.12	9.4

*** Significant at P≤0.001.

‡ Un= unamended; CF= chemical fertilizer; CrF_{low}, CrF_{medium} and CrF_{high}= Cricket feces at rates of 3.125, 6.25 and 12.5 Mg ha^{-1}, respectively and -Nm and +Nm= without and with neem seed extract.

‡ Means within the same column followed by the same letter are not significantly different at P≤0.05 (Tukey's HSD test).

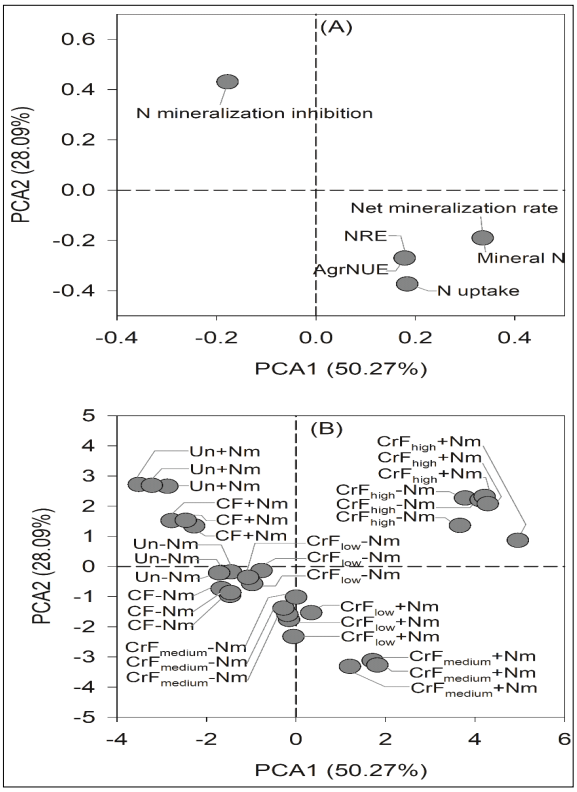

Fig 1: Eigenvector values of (A) soil N status, N use efficiencies and amaranth's shoot dry weight as affected by the combined uses of cricket feces and neem seed extract and (B) the factor scores of amendment treatments; where Un= unamended; CF= Chemical fertilizer; CrF_{low}, CrF_{medium} and CrF_{high}= Cricket feces at rates of 3.125, 6.25 and 12.5 Mg ha^{-1}, respectively and -Nm and +Nm= Without and with neem seed extract; NRE= N recovery efficiency; AgrNUE= Agronomic N use efficiency.

corroborated by the PCA results (Fig 1). The NRE and AgrNUE existed in the opposite PCA-quadrant to N mineralization inhibition but in the same quadrant with mineral N and net N mineralization rate (Fig 1A). In addition, CF+Nm (Fig 1B) was deposited on a similar quadrant with N mineralization inhibition (Fig 1B). This result was not the case for CF-Nm. The results indicated that CF+Nm decreased NRE and AgrNUE. Nitrification inhibition might stimulate N loss via gases such as NH_3 volatilization. Soares *et al.* (2012) demonstrated increased NH_3 volatilization by using dicyandiamide as a nitrification inhibitor in an acidic soil. Loss of N from the soil system in this study was verified, in part, by significantly lower mineral N in CF+Nm than CF-Nm (Table 3).

CONCLUSION

This study has demonstrated that neem seed extract could inhibit the nitrogen mineralization only in unamended and chemically fertilized soils and *vice versa* for the cricket feces amended soils. Nitrogen use efficiencies of amaranth decreased only in the combined uses of neem seed extract

with unamended and chemical fertilizer. Assessing the residual content of ingredients relating to the inhibitory effect of the neem extract on N mineralization is necessary to understand better the ineffectiveness of the extract in cricket feces amended soils.

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