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SHORT COMMUNICATION

Development of a Botanical Formulation Using *Cinnamomum verum* and *Pongamia pinnata* Leaves for Suppression of Nitrification in Soil

A.M. Dias[1] and W.S. Dandeniya[2]*

[1]*Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, 20400, Sri Lanka.*
[2]*Department of Soil Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, 20400, Sri Lanka.*

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Dandeniya, W.S.
https://orcid.org/0000-0003-0795-3923

ABSTRACT

There is a growing interest in controlling nitrification by using botanical nitrification inhibitors to enhance the nitrogen (N) fertilizer use efficiency. The main objective of this study was to develop a botanical formulation (BF) with *Cinnamomum verum* (cinnamon) and *Pongamia pinnata* (karanda) leaves to suppress the nitrification rate of two soils (Red Yellow Podzolic (RYP) and Reddish-Brown Earth (RBE)) commonly used for vegetable cultivation in Sri Lanka. A BF was prepared using dried and powdered leaves of cinnamon and karanda, biochar and corn flour. The two soils were treated with BF at three rates (0, 5 and 10%, w/w) and the potential nitrification rate (PNR) was determined using the shaken slurry method in a laboratory incubation. The obtained data were analysed by Statistical Analysis Software (SAS) adopting a two factor factorial design. The BF application significantly delayed ($P<0.05$) nitrate formation in both soils. The RBE soil had significantly lesser ($P<0.05$) PNR compared to RYP soil. Application of 5 and 10% BF, significantly reduced PNR in both soils at 4 h of incubation. However, only a 10% BF application rate remained effective in suppressing PNR at 21 h of incubation. Results indicate the possibility of using dried, powdered leaves of cinnamon and karanda for suppressing nitrification in soil. Further studies are required to investigate the effect of developed BF on soil biological properties.

*Corresponding author- warshisd@agri.pdn.ac.lk

Nitrogen (N) is an essential nutrient required for plant growth, development and reproduction. Despite N is one of the most abundant elements on the earth, N deficiency is the most common nutritional problem affecting plant growth (Vakilian and Massah, 2012) because N from the atmosphere and earth's crust is not directly available for plant uptake. Therefore, as a solution, farmers apply N fertilizers to fulfill the crop N requirement. However, N applied with fertilizers could be loss from soil through three processes; leaching, denitrification and volatilization. Further, N application rates by farmers to their agricultural fields often exceed the actual crop use. Therefore, managing N inputs and losses in agricultural systems is critical to reduce the burden of agriculture on the environment (Thomsen *et al*., 1993).

To reduce the loss of N from soil to the environment, techniques should be used to keep applied N in the topsoil and increase the fertilizer use efficiency of crops in agricultural lands. Most organic and inorganic N fertilizers supply ammonium (NH_4^+) to soil and this NH_4^+ is converted to nitrate (NO_3^-) through a microbiologically mediated process called nitrification. Nitrate is more mobile in the soil environment than NH_4^+, and leaching of NO_3^- contributes to the contamination of surface and groundwater (Thomsen *et al*., 1993). Therefore, efforts should be taken to control nitrification to enhance N-fertilizer use efficiency in agriculture. The use of nitrification inhibitors along with N-fertilizers is one of the measures of suppressing nitrification in soil. Although there are synthetic nitrification inhibitors these products are expensive and not available in the market for the farmers in Sri Lanka. According to Nawarathna *et al*. (2021), there are locally available plant materials such as cinnamon, karanda, pepper, and nutmeg that could be used as nitrification inhibitors. Therefore, this study was conducted to develop a botanical formulation (BF) from locally available plant materials such as *Cinnamomum verum* and *Pongamia pinnata* to suppress the nitrification in two agricultural soils with different soil properties.

The two soils used in this experiment were collected from vegetable cultivated fields in Nuwara Eliya and Maha Illuppallama, which belong to WU3 and DL1 agro-ecological regions of Sri Lanka, respectively. The soil from Nuwara Eliya is a Typic Paleudults (an Ultisol), which belongs to the Red Yellow Podzolic (RYP) great soil group according to the local classification (Mapa and Somasiri, 1999). The soil from Maha Illuppallama is a Typic Rhodustalf (an Alfisol) which belongs to the Reddish Brown Earth (RBE) great soil group according to the local classification (Dassanayaka *et al*., 2020). All laboratory analyses were conducted at the Department of Soil Science, Faculty of Agriculture, University of Peradeniya. Soil samples were air-dried and sieved through a 2 mm sieve prior to experiments. As basic soil properties, soil texture, pH, Electrical Conductivity (EC), available N, and total N were analysed in both soils following standard protocols (Dharmakeerthi *et al*., 2007).

Botanical formulations (BF) were developed using mature leaves of *C. verum* (cinnamon) and *P. pinnata* (karanda) that were collected and cleaned with distilled water on the same day. Leaves were dried at 60 °C for 24 h and processed into a fine powder separately using grinding mill. Then, BF pellets were prepared manually by using dried and powdered cinnamon and karanda leaves by adding paddy-husk biochar as the inert material and corn flour as the binding agent to the leaf powder mixture. Cinnamon and karanda leaf powder were used in equal amounts. Pellets were oven dried at 60 °C until a constant weight was obtained and stored in air tight containers. The total weight of both cinnamon and karanda leaf powder in the pellets was 57 % and the total N content was 1.77 % (w/w). The ability of the product to suppress nitrification was determined by performing **a** potential nitrification rate (PNR) assay for the two soils separately with three rates of pellet application; 0, 5 and 10% (w/w) in triplicates. The PNR assay was performed according to the shaken and slurry method (Hart *et al*., 1994). The method in brief, 15 g of field moist soil was weighed into a 250 ml Erlenmeyer flask and 100 ml of 1.5 mM NH_4^+ containing phosphate-buffer medium pH adjusted to 7.2 (27.22 g KH_2PO_4, 34.84 g

K_2HPO_4 and 6.607 g NH_4SO_4 in 1 L) was added to soil. Pellet was added to the soil at above mentioned rates. Then, the samples were shaken at 180 rpm for 24 h in an orbital shaker allowing the aerobic condition during incubation at 26 °C. The slurry was sampled four times during the 24-h period (at 2-, 4-, 21- and 24-h into incubation) and analysed for NO_3^- colourimetrically (Cataldo *et al*., 1975). The NO_3^- formation rate per unit of soil was calculated (mg- NO_3^- -N formed/kg soil/h).

Data were analysed using Statistical Analysis Software (SAS) adopting a two-factor factorial design using soil type (two soils; RYP and RBE) and pellet ratio (0, 5 and 10 %) as the grouping factors. The significance of treatment effects was analysed at P<0.05 level. When the main effects or the interactions between main effects were significant on measured parameters the mean comparison was performed with Duncan's Multiple Range Test at P<0.05.

The potential nitrification rate is a measure used to identify the rapidity of nitrification with the provision of an ample amount of NH_4^+ under optimum conditions for nitrification (Hart *et al*., 1994). Based on the results PNR of RYP soil is significantly greater (P<0.05) than that of RBE soil (Figure 1). Nawarathna *et al*. (2019) reported that intensively cultivated RYP soils in Nuwara Eliya have PNR less than 3 mg NO_3^--N/kg dry soil/h. Therefore, the observed PNR in this study is within the ranges reported in the literature. However, Gnanavelrajah and Kumaragamage (1998) have reported that normally RYP soil is having low nitrification rate probably due to its low pH value. De Boer and Kowalchuk (2001) also reported that the available isolates of ammonia-oxidizing bacteria (AOB) were not much tolerant to soil acidic conditions and their nitrification rates also decreased dramatically as pH decreased. Based on a study conducted with tea cultivated acidic soils, PNR ranged from 0.05 to 1.32 mg NO_3^--N/kg dry soil/h (Yao *et al*., 2011). The high potential for nitrification observed in this study may be due to the adaption of nitrifiers in these cultivated soils that receive a lot of NH_4^+ ions through N fertilization (Nawarathna *et al*., 2019).

The treatment effect on PNR was more prominent at 4 h of incubation compared to 21 h of incubation (Figure 1). The application of 5 and 10% BF, significantly reduced PNR in both soils at 4 h into incubation (Figure 1A). However, the treatment effect was less prominent towards 21 h into incubation (Figure 1B). The change in effectiveness over time could be due to changes in other biological processes, especially the activity of heterotrophic bacteria in response to the input of organic substrates with the formulation (Nawarthna *et al*., 2021). Because, the addition of high carbon (C) substrate to soil during laboratory incubation stimulates the rapid growth of heterotrophic bacteria and results in lower rates of net nitrification (Hart *et al*., 1994). The application of BF significantly delayed (P<0.05) the formation of nitrate in both soils (Figure 2).

Table 1: **Selected chemical and physical characteristics of soil used for the experiment**

Parameter	RYP	RBE
Soil texture	Sandy loam (sand:silt:clay = 74:17:9)	Sandy clay loam (sand:silt:clay = 69:11:20)
Soil pH	4.9±0.01	7.3±0.02
Electrical Conductivity (dS/m)	0.075±0.01	0.031±0.01
Available N (mg/kg)	98±0.02	70±0.01
Total N (%)	0.28±0.02	0.14±0.01
Organic matter (%)	2.31±0.11	1.15± 0.05

Average (n= 3) ± Standard deviation

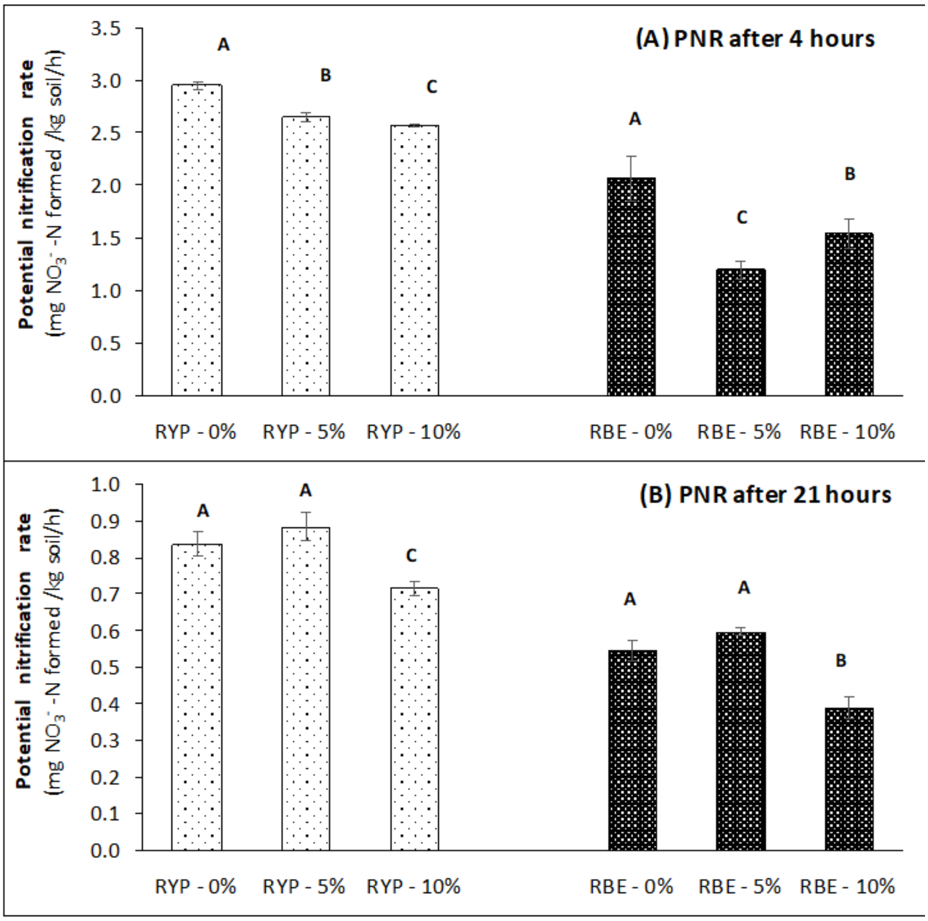

Figure 1: The potential nitrification rates (PNR) of RYP and RBE soils as affected by the application of botanical formulation (BF) at different application rates [0, 5 and 10% (w/w)] at 4 h (A) and 21 h (B) into incubation. Error bars represent standard deviation. The same capital letter above each bar for a given soil indicates that the means are not significantly different (*P*>0.05).

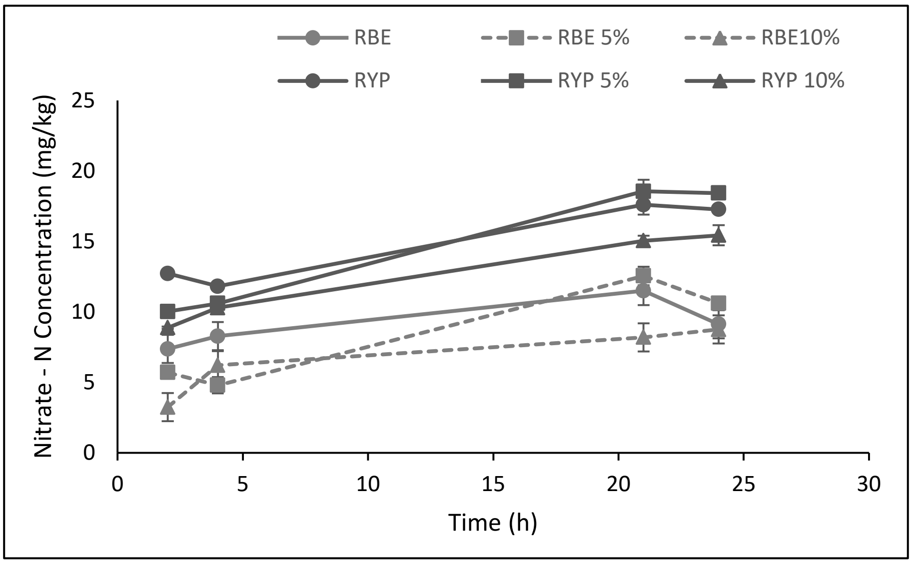

Figure 2: Nitrate concentration in two soils (RBE and RYP) during incubation as affected by the application of botanical formulation (BF) at 0, 5 and 10% rates. Error bars represent standard deviation.

As indicated by Norton and Ouyang (2019) the plant-based natural botanical nitrification inhibitors can affect both ammonia-oxidizing bacteria (AOB) such as *Nitrosomonas* and nitrite-oxidizing bacteria (NOB) such as *Nitrobacter*, since these materials are capable of reducing the abundance and/or retarding the activity of AOB and NOB. The results from the present study suggest that there could be a soil-to-soil variation in the effectiveness of botanical products. Further, Nawarathna *et al*. (2021) have found that the leaf powder of cinnamon can suppress the growth of AOB. Moreover, karanda can suppress the activity of AOB and retarding nitrification (Prasad *et al*., 1989; Nawarathna *et al*., 2021). Therefore, cinnamon and karanda can be used as good botanical nitrification inhibitors. Incorporation of these plant powders which are capable of inhibiting nitrification or delaying the conversion of NH_4^+ into NO_3^- into vegetable cultivations in the country may help retain N in these soil systems while increasing the soil N pools. Moreover, it will help reduce NO_3^- leaching losses to groundwater while alleviating the N fertilization.

Overall, the results indicate that the botanical formulation that was developed using dried powdered leaves of cinnamon and karanda could reduce the rate of nitrification under laboratory conditions. The product needs to be tested under field conditions because the effectiveness in inhibiting nitrification seems to change with application rate, time and soil type.

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