Chemosphere 76 (2009) 293–299

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Effect of pest controlling neem (*Azadirachta indica* A. Juss) and mata-raton (*Gliricidia* sepium Jacquin) leaf extracts on emission of green house gases and inorganic-N content in urea-amended soil

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ARTICLE INFO

Article history:
Received 31 October 2008
Received in revised form 25 March 2009
Accepted 3 April 2009
Available online 7 May 2009

Keywords:
Bioinsecticide
Dynamics of C and inorganic N
Lambda-cyhalothrin
Soil water content

ABSTRACT

Extracts of neem (*Azadirachta indica* A. Juss.) and *Gliricidia sepium* Jacquin, locally known as 'mata-raton', are used to control pests of maize. Their application, however, is known to affect soil microorganisms. We investigated if these extracts affected emissions of methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O), important greenhouse gases, and dynamics of soil inorganic N. Soil was treated with extracts of neem, mata-raton or lambda-cyhalothrin, used as chemical control. The soil was amended with or without urea and incubated at 40% and 100% water holding capacity (WHC). Concentrations of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) and emissions of CH_4, CO_2 and N_2O were monitored for 7 d. Treating urea-amended soil with extracts of neem, mata-raton or lambda-cyhalothrin reduced the emission of CO_2 significantly compared to the untreated soil with the largest decrease found in the latter. Oxidation of CH_4 was inhibited by extracts of neem in the unamended soil, and by neem, mata-raton and lambda-cyhalothrin in the urea-amended soil compared to the untreated soil. Neem, mata-raton and lambda-cyhalothrin reduced the N_2O emission from the unamended soil incubated at 40%WHC compared to the untreated soil. Extracts of neem, mata-raton and lambda-cyhalothrin had no significant effect on dynamics of NH_4^+, NO_2^- and NO_3^-. It was found that emission of CO_2 and oxidation of CH_4 was inhibited in the urea-amended soil treated with extracts of neem, mata-raton and lambda-cyhalothrin, but ammonification, N_2O emission and nitrification were not affected.

1. Introduction

Extracts of neem (*Azadirachta indica* A. Juss.) are being extensively used to control pests (Schmutterer, 1985; Akhtar and Mahmood, 1997; Amadioha, 2000; Michereff et al., 2008). The activity of neem has been attributed to the more than 300 components isolated from the different parts of the tree (Devakumar and Dev, 1993). Azadirachtin, a complex limonoid, found in the neem seeds is the main component with antifeedant and toxic effects in insects (Mordue and Nisbet, 2000; Schaaf et al., 2000). Other limonoid and sulphur-containing compounds with repellent characteristics are found in the leaves, flowers, bark and roots (Mordue and Nisbet, 2000). Neem, however, originates from India, and could easily deteriorate local ecosystems. In a previous experiment, *Gliricidia sepium* (Jacquin), also known as 'mata-raton', was used as an alternative to control pests on maize (Montes-Molina et al., 2008a). Little is known about the components in mata-raton that are bioactive towards pests, but it is a known insecticide and its leaves contain triterpene saponins (Rastrelli et al., 1999; Rojas et al., 2006). Leaf extracts of mata-raton decreased damage to maize and increased yields, although less than leaf extracts of neem, compared to untreated plants in a field experiment (Montes-Molina et al., 2008b).

Extracts of neem, however, are also known to affect soil microorganisms. Gopal et al. (2007) studied the effect of granules containing 10% azadirachtin, i.e. alcoholic extract of neem seed kernel mixed with China clay, on the population of bacteria, actinomycetes, fungi, *Azotobacter* sp. and nitrifying bacteria. They found that azadirachtin exerted a negative effect on the microbial communities in the initial 15 d, but not on *Azotobacter* sp. Montes-Molina et al. (2008a) found that the amount of nodules formed on common bean (*Phaseolus vulgaris* L.) was lower in soil treated with neem leaf extracts than in untreated soil. It has also been shown that neem oils can be used as a nitrification inhibitor (Kumar et al., 2007), that seed kernel powder retards urease and

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doi:10.1016/j.chemosphere.2009.04.007

nitrification activities in different soils (Mohanty et al., 2008) and neem cake reduces emission of N_2O and CH_4 (Malla et al., 2005). Neem leaf extracts could thus have an important effect on dynamics of C and N, greenhouse gas emissions and nutrient availability in soil. Extracts of mata-raton have antimicrobial activity, so they might also affect soil microorganisms (Rojas et al., 2006).

We investigated the effects of leaf extracts of neem and mata-raton on emissions of CH_4, CO_2 and N_2O, and dynamics of inorganic N in unamended soil. Soil was incubated at 40% water holding capacity (WHC) and 100% WHC added with or without urea. Urea is the most commonly used N fertilizer in Mexico and when added to soil will increase nitrification. Lambda-cyhalothrin (Karate) was used as a chemical control. Concentrations of NH_4^+, NO_2^-, NO_3^- and emissions of N_2O, CO_2 and CH_4 were monitored in an aerobic incubation for 7 d. The objective of the study was to investigate the effect of neem and mata-raton extracts on dynamics of C and N soil amended with or without urea.

2. Materials and methods

2.1. Leaf extract preparation

Twenty mature neem (*A. indica*) and 20 mata-raton (*G. sepium*) trees were selected at random at the South-East of Tuxtla Gutierrez in the state of Chiapas in the South of México at 16° 46′ 24.21″ latitude north and 93° 10′ 22.48″ longitude west. The average altitude of the area is 600 m above sea level and characterized by a mean annual temperature ranging from 27 to 33 °C with an average annual precipitation of 400 mm without exceeding 600 mm, mainly from May to October. Five hundred g leaves were sampled from each tree and pooled. As such, ten kg of leaves was collected. Neem leaves were at least three months old and had lost their flexibility so that insects, i.e. ants, did not damage them any more. One hundred g of fresh leaves was washed with water to clean them, cut in 2 mm^2 squares, added to 1 dm^3 water, left in the dark for 72 h, which was sufficient to extract most of the active components of neem and mata-raton. The solution was then filtered and made up to 3 dm^3 with water (Montes-Molina et al., 2008a). Extraction procedures were kept as simple as possible so as to aid the farmer in its preparation. Lambda-cyhalothrin which served as a chemical insecticide was obtained from Syngenta, Willmington, Del., USA.

2.2. Soil sampling site and characteristics of soil

Soil was collected in Otumba, State of Mexico, Mexico, (N.L. 19°42′, W.L. 98°49′). Its average altitude is 2349 m above sea level and characterized by a sub-humid temperate climate with a mean annual temperature of 14.8 °C and average annual precipitation of 577 mm mainly from June through August (http://www.inegi.gob.mx). The soil is sandy loam with pH 7.6 and electrolytic conductivity (EC) 1.15 dS m^{-1}, an organic C content of 7.2 g C kg^{-1} soil, inorganic C content of 661 mg kg^{-1}, and 1 g kg^{-1} of total N. 27.1 mg NO_3^- kg^{-1} dry soil. 1.3 mg NO_2^- kg^{-1} dry soil, and 6.0 mg NH_4^+ kg^{-1} dry soil, and WHC of 564 g kg^{-1} dry soil. The area is mainly cultivated with maize and common bean, receiving a minimum amount of inorganic fertilizer without being irrigated (http://www.inegi.gob.mx). Soil was sampled at random by augering the 0–15 cm top-layer of three plots of approximately 0.5 ha. The soil from each plot was pooled and as such a total of three soil samples were obtained.

2.3. Experimental set-up and treatments

Soil samples were taken to the laboratory, sieved (<5 mm), air-dried and characterized. Hundred and ninety-two sub-samples of 10 g dry soil from each plot were added to 120 mL flasks. Forty-eight samples were amended with 105 μL of leaf extract (considered the NEEM treatment), 48 with 105 μL of mata-raton extract (considered the MATA-RATON treatment), 48 with 105 μL lambda-cyhalothrin (considered the CHEMICAL treatment) and the rest was left unamended (considered the CONTROL treatment). Twelve sub-samples from each treatment were adjusted to 40% or 100% WHC by adding distilled H_2O, while 12 were adjusted to 40% or 100% WHC by and amended with a 0.35 M urea solution. The amount of urea added was such that 100 mg N kg^{-1} soil was added. As such, 16 treatments were generated, i.e. soil at two different water contents, amended with or without urea and added with neem leaf extract, mata-raton leaf extract, insecticide or left unamended.

Three flasks were chosen at random from each treatment and plot and soil was extracted for inorganic-N with 100 ml 0.5 M K_2SO_4 solution. The samples were shaken for 60 min and filtered through Whatman No 42 paper®. This provided zero-time samples. The extracts were stored at −20 °C pending analysis. The flasks were stoppered with Suba-seals and incubated at 25 °C for 7 d. An additional nine flasks without soil were sealed and served as controls to account for the N_2O, CO_2 and CH_4 in the atmosphere. After 1, 3 and 7 d, three flasks were selected at random from each treatment and the headspace was analyzed for CH_4, CO_2 and N_2O. The flasks were opened and the soil was analyzed for inorganic N as described earlier.

2.4. Soil chemical analyses

Soil pH was measured in 1:2.5 soil-H_2O suspension using a glass electrode (Thomas, 1996). The EC was determined in a 1:5 soil/H_2O suspension as described by Rhoades et al. (1989). The organic C in soil was measured in a total organic carbon analyzer TOC-V_{CSN} (SHIMADZU, USA). Inorganic C in soil was determined by adding 20 ml 1 M HCl solution to 2 g air-dried soil and trap the CO_2 evolved in 20 ml 1 M NaOH. Total N was measured by the Kjeldahl method using concentrated H_2SO_4, K_2SO_4 and $CuSO_4$ to digest the sample (Bremner, 1996). The NH_4^+, NO_2^- and NO_3^- in the K_2SO_4 extracts were determined colourimetrically on a San Plus System – SKALAR automatic analyzer (Mulvaney, 1996). The WHC was measured as described by Gardner (1986). Soil particle size distribution was determined by the hydrometer method as described by Gee and Bauder (1986).

The headspace volume of each flask was sampled and analyzed according to Holland et al. (1999). A Shimadzu gas chromatograph GC-14B fitted with an electron capture detector was used for the measurement of N_2O and CO_2 (Holland et al., 1999). A Porapak Q column used to separate N_2O and CO_2 from the other gases with the carrier gas He flowing at a rate of 55 ml min^{-1} was maintained at 35 °C. The amount of CH_4 was determined with an Agilent 4890D gas chromatograph fitted with a flame ionization detector. A Porapak Q column (80/100 12′ × 1/8″ × 0.085″) was used to separate CH_4 from the other gases with the carrier gas He flowing at a range of 25 ml min^{-1}. Injection, detection and column-oven temperatures were set at 100 °C, 310 °C, and 32 °C, respectively. The CH_4, CO_2 and N_2O dissolved in soil–water was accounted for considering their specific solubility (Moraghan and Buresh, 1977).

2.5. Statistical analysis

Emission of CO_2 and N_2O was regressed on elapsed time using a linear regression model, which was forced to pass through the origin but allowed different slopes (production rates) for each treatment. This approach is supported by theoretical considerations that no CO_2 and N_2O was produced at time zero and the control without soil accounted for the CO_2 and N_2O in the atmosphere.