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Short Communication

Microbial Influence on Release of Acetylene and Ethylene from Calcium Carbide and Its Impact on Nitrification Inhibition

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ABSTRACT

Incubation studies were conducted to evaluate the microbial influence on the release of acetylene (C_2H_2) and ethylene (C_2H_4) in soil amended with encapsulated calcium carbide (ECC) by using gas chromatography and monitoring C_2H_2 as nitrification inhibitor. The GC-FID analysis revealed that under non-sterilized conditions ECC produced 542.1 µmol of C_2H_2 kg^{-1} soil over a period of 60 days. Under sterilized conditions 796.5 µmol C_2H_2 kg^{-1} soil was produced over a period of 60 days. Under non-sterilized conditions C_2H_2 was reduced gradually to C_2H_4 over a period of time and 44.8 µmols of C_2H_4 were recorded on 60th day of the incubation, however, under sterilized conditions no C_2H_4 was observed. This implies that conversion of C_2H_2 to C_2H_4 is a strictly biological process as no C_2H_4 was detected under sterilized conditions. The second study revealed that oxidation of NH_4^+ was reduced by ECC and NH_4^+-N concentration was greater (up to 82%) by the application of ECC. Similarly NO_3^--N was 79% greater in 30 mg N alone compared with ECC.

Key Words: Acetylene; Ethylene; sterilization; Calcium carbide; Encapsulated

INTRODUCTION

Undesirable effects of fertilizer use on increased production of N_2O can be grappled with by agricultural management with out affecting production. The main aim of this experiment was to evaluate calcium carbide (CaC_2) as a source of C_2H_2, which has been proved by a number of workers as a potent nitrification inhibitor (Thompson, 1996; Ahmad *et al.,* 2008). Further more, C_2H_2 is claimed to be converted into C_2H_4 by soil indigenous microbes, which acts as a plant growth regulator (Arshad & Frankenberger, 2002: Ahmad *et al.,* 2008). Calcium carbide has recently been reported to increase the concentration of the plant hormone C_2H_4 in soil air as a result of microbial reduction of C_2H_2 into ethylene glycole and ethylene oxide (Arshad & Frankenberger, 2002). C_2H_2 inhibits the activity of ammonia-oxidizing enzyme involved in the nitrification (Porter, 1992; Chen *et al.*, 1994), resulting in inhibition of nitrification and denitrification and increased N use efficiency (Sahrawat, 1989; Keerthisinghe *et al*., 1996; Thompson, 1996; Aulakh *et al*., 2001). C_2H_4 is a potent plant growth regulator, involved in almost all the phases of growth and development of plant (Abeles *et al*., 1992; Muromtsev *et al*., 1995; Arshad & Frankenberger, 2002; Ahmad *et al.,* 2008). However, the use of C_2H_4 for the improvement of agricultural production has been limited because of its gaseous nature and therefore it has been difficult to use it directly to soil in the field. Various compounds have been identified which promote C_2H_4 concentration in soil air (Bibik *et al.*, 1995; Akhtar *et al*., 2005; Khalid *et al*., 2006b). Since microorganisms can derive C_2H_4 from a variety of compounds, it is highly likely that the presence of a substrate (s) in the rhizosphere can influence the production of physiologically active concentrations of C_2H_4.

Ethylene releasing preparation under the trade name of Retprol was introduced by the Russian Scientists (Muromtsev *et al.,* 1995; Bibik *et al.,* 1995) as a slow releasing compound. This product is a preparative form of CaC_2 that decomposes in soil. In recent years many workers have reported the involvement of microbes in the reduction of C_2H_2 to C_2H_4 (Arsahd & Frankenberger, 2002; Bandyopadhyay, 2005; Ahmad *et al.,* 2008).

MATERIALS AND METHODS

An incubation study was conducted under laboratory conditions to evaluate the microbial influence on the production of C_2H_2 and C_2H_4 from CaC_2. Sandy clay loam soil was collected from the upper layer of experimental fields at Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad. The soil was air-dried, ground, sieved and analyzed for physico-chemical properties following the procedures and methods described by USDA (1954). The soil had pH, 7.8; sand, 49.68%; silt, 28.74%; clay, 21.58%; WHC (water holding capacity), 32.2%; organic carbon,

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0.44% and total N, 0.05%. Calcium carbide (27% i.e., Ningxia National Chemical Group Co. Ltd., China) was purchased from the local market and was ground to powder. Powdered calcium carbide was encapsulated in a matrix comprising of polyethylene, starch, soil and wheat straw and the formulation thus obtained was extruded and the noodles cut into 3-5 mm pieces.

Release of acetylene and ethylene. The C_2H_2 and C_2H_4 produced in soil amended with ECC, was determined by gas chromatography. 100 g of the same processed soil were taken in a 250 mL Erlenmeyer flask at 60% WHC. The formulation at the rate of 30 mg kg^{-1} soil was placed in the center and the flasks were capped with rubber corks and incubated at 30^oC. For non-biological production of C_2H_2 and C_2H_4, soil was sterilized at 121^oC for 1 h on alternate days and calcium carbide was added under sterilized conditions. There were three replications and controls were also run.

Release of C_2H_2 and C_2H_4 gases was studied over a period of 60 days by gas chromatograph (Carlo-Erba FVS-2300) following the protocol described by Arshad *et al.* (2004). The Gas chromatogram was run isothermically and capillary column packed with Porapak N was used under the following conditions: carrier gas, N_2 (13 mL min^{-1}); H_2 flow rate, 33 mL min^{-1}; air flow rate, 360 mL min^{-1}; sample volume, 1 mL; column temperature, 70^oC; detector temperature, 200^oC. Standards were run and C_2H_2 and C_2H_4 concentrations were determined by comparison. Ethylene identification was based on the retention time compared with a C_2H_4 standard (purity, 99.9%).

Evaluation of calcium carbide as an inhibitor for nitrification. Plastic beakers (2 kg capacity) were filled with one kg soil. Three rates of N (0, 15, 30 mg kg^{-1} soil) as urea were dissolved in distilled water and uniformly mixed with the soil. Each treatment was applied in triplicate following CRD design. ECC @ 0, 15 and 30 mg kg^{-1} soil was placed 6 cm deep in the center of each beaker, so that C_2H_2 gas could uniformly be diffused to all the directions. Calcium sulfate ($CaSO_4$) equivalent to the amount of calcium in CaC_2 was added in control to mitigate the deficiency of calcium. Distilled water was used to maintain the soil moisture near field moisture capacity (60% MC) up to six weeks from the start of the experiment. Tops of the beakers were kept open, while their sides were wrapped with aluminum foil. The beakers were placed in the laboratory (25±5°C). After six weeks, the contents of each beaker were taken out and mixed thoroughly. Moist soil, equivalent to 10 g dry weight, was extracted for 1 h with 100 mL of 2 M KCl solution containing 15 µm phenylmercuricacetate and filtered through Whatman No. 42 filter papers. The filtrate was then analyzed for NH_4^+-N by Indophenol Blue method and NO_3^--N by a modified Griss-Ilosvay method (Kenney & Nelson, 1982).

RESULTS

Results revealed that under sterilized condition, the release of C_2H_2 was very slow (Fig. 1a), as compared to non-sterilized conditions. Maximum C_2H_2 (108.69 µmol kg^{-1} soil) was produced on 56^{th} day of incubation and minimum (4.6 µmol kg^{-1} soil) on the day of incubation. After 56 days decline in C_2H_2 concentration started and on 70^{th} day 49.78 µmol kg^{-1} soil, C_2H_2 was observed. Under non-sterilized conditions (Fig. 1b), C_2H_2 showed almost the same pattern as under sterilized condition. Data indicates that with the passage of time the production of C_2H_2 decreased and after 28 days a sharp decline occurred. There was no impact of sterilized and non-sterilized conditions on C_2H_2 production indicating that sterilized or non-sterilized conditions have no role in the C_2H_2 production behavior in soil, because it is a chemical reacti; however, the degradation of formulation was quick in this case due to the presence of soil microbes, which caused decay of the ECC due to the presence of starch and wheat straw.

Under non-sterilized conditions, during early days C_2H_4 production (Fig. 1c), was not observed; however, on 7^{th} day onwards, C_2H_4 was detected in the treatments, which increased with the passage of time and maximum C_2H_4 8.92 µmol kg^{-1} soil was recorded on 60^{th} day of incubation. Soil native C_2H_4 was also monitored that increased with time. Soil microorganisms, present under non-sterilized conditions might have contributed towards conversion of C_2H_2 to C_2H_4 in CaC_2 treated soil as well as native C_2H_4 detected in control, because under sterilized condition no C_2H_4 was detected even in the presence of C_2H_2. Under sterilized conditions, no C_2H_4 was detected (Fig. 1d), in CaC_2 treated as well as in control soil. This indicates that microbial presence is necessary for the reduction of C_2H_2 to C_2H_4. As microbes do not sustain such environment so the reduction of C_2H_2 to C_2H_4 did not occur.

The effect of ECC on the oxidation of NH_4^+ to NO_3^- in the soil is shown in Fig. 2. The ECC decreased conversion of NH_4^+ to NO_3^- significantly ($P \leq 0.05$). In control NO_3^- content was (2%) greater than NH_4^+ content. The treatment where ECC @ 15 mg kg^{-1} soil was applied in the absence of N showed 33% decrease in NO_3^+ content over NH_4^+. Contrary to this, N @ 15 mg kg^{-1} soil increased NO_3^+ content up to 40% over NH_4^+. The treatment having N and ECC in the ratio of 1:1 showed that NH_4^+ content was greater than NO_3^+ up to 35%. Similarly ECC @ 30 mg kg^{-1} soil increased NH_4^+ content up to 82% and N at the same rate in the absence of ECC decreased NH_4^+ content up to 79% over NO3. 44% more NH_4^+ observed in the treatment, where ECC and N were applied @ 30 mg kg^{-1} soil.

DISCUSSION

Commercial exploitation of CaC_2 to induced responses in plants is a new idea. Calcium carbide has been used in many agricultural applications by taking advantage of its C_2H_2 releasing property and its action was studied as nitrification inhibitor by different researchers (Aulakh *et al.,* 2001; Randall *et al*., 2001). Different sources like commercial C_2H_4 gas, ACC, ethephon and ethereal are used

Fig. 1. Production of acetylene and ethylene from encapsulated calcium carbide under sterilized and non-sterilized conditions

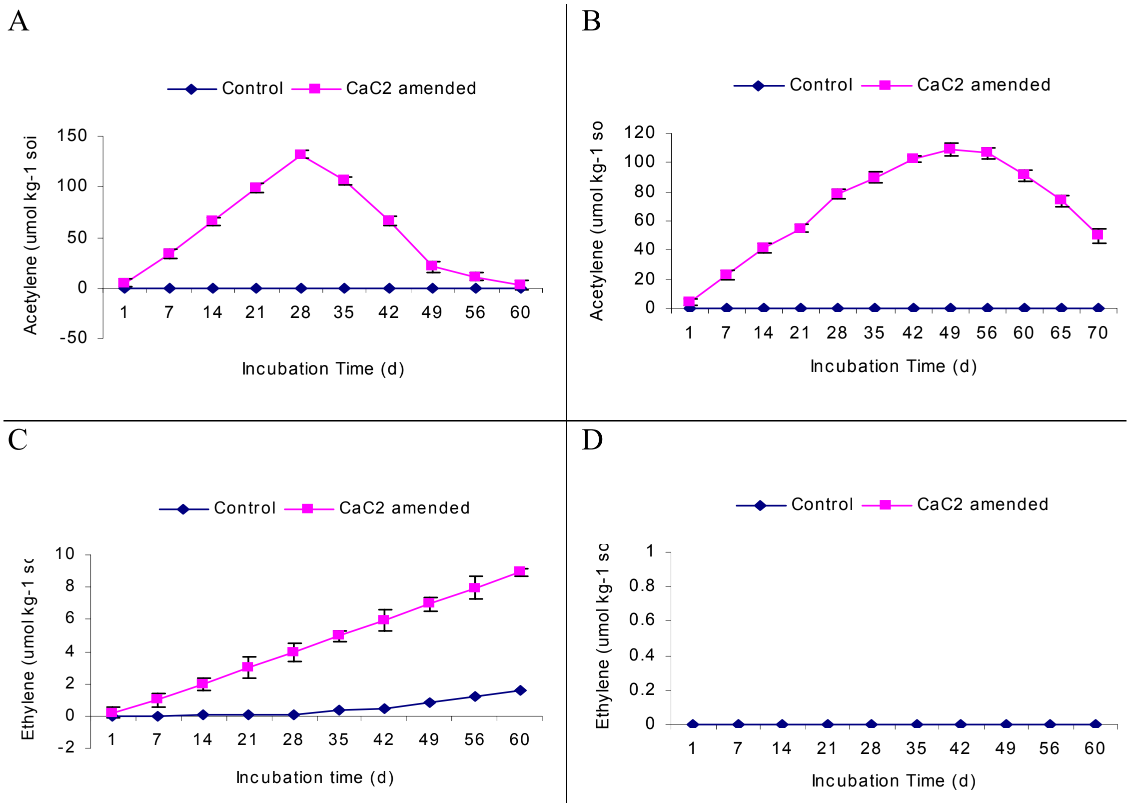
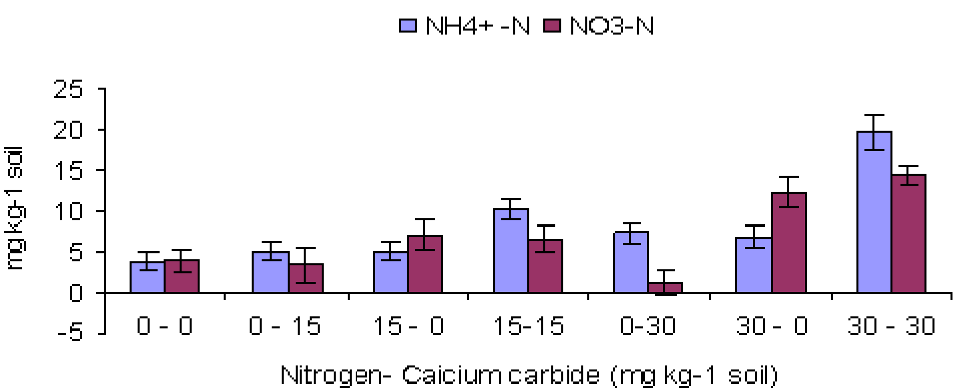


Fig. 2. NH_4^+-N and NO_3^--N contents of soil after the application of encapsulated calcium carbide under laboratory conditions



which chemically or enzymatically provide C_2H_4 to plants. Calcium carbide when added to soil absorbs moisture and releases C_2H_2 gas, which is a potent nitrification inhibitor (Freney *et al*., 1992; Ahmad *et al.,* 2008), is converted to C_2H_4 in rhizosphere or within plants, enzymatically (Arshad & Frankenberger, 2002). From this study it can be concluded that CaC_2 can be a source of C_2H_2, which ultimately is converted to C_2H_4 after a lag period of 7 days under laboratory conditions.

The application of ECC significantly ($P \leq 0.05$) suppressed the oxidation of NH_4^+-N to NO_3^--N in a soil amended with N fertilizer (urea). This suggests that CaC_2 could be used as a nitrification inhibitor (Freney *et al*., 1992). The suppressive impact of CaC_2 on NH_4^+-H oxidation is also supported by the findings of other researchers (Sahrawat, 1989; Crawford & Chalk, 1992; Porter, 1992). The results of the present study revealed that encapsulated CaC_2 plus N fertilizer had significantly ($P \leq 0.05$) higher NH_4^+ contents with compared to NO_3^--N in soil for a period that may go up to six weeks or more than this as demonstrated in the study. These positive effects of CaC_2 in the presence of N fertilizer could be attributed to physiologically active levels of C_2H_2, which acted as nitrification inhibitor.

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