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RESEARCH ARTICLE

Nitrification Inhibition Potential of *Brachiaria humidicola*

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Abstract An incubation experiment was conducted to determine the nitrification inhibition potential of *Brachiaria humidicola* (*B. humidicola*) and their effect on nitrification process. The pots soil was mixed 2 mg of nitrogen through ammonium sulphate. Seven treatments were evaluated viz. control, four root extracts of *B. humidicola* called as biological nitrification inhibitors (BNIs) (i.e., 70 % ethyl alcohol, 40 % ethyl alcohol, phosphate buffer solution and 2 M KCl salt solution extracts) and two standard chemical inhibitors i.e. dicyandiamide and neem oil coating. The amount of NH_4^+-N was reduced 20.66–11.91 μg g^{-1} soil and NO_3^--N increased 28.89–31.18 μg g^{-1} soil from 14th to 22nd day time interval. Percent nitrification inhibition was more in BNIs (70 and 40 % alcohol extract) treated soils compared to plant based and synthetic nitrification inhibitors. The nitrification inhibition by *B. humidicola* also varied it was maximum (64.71 %) observed at 14th day over 22nd day (49.63 %).

Keywords *Brachiaria humidicola* · BNIs · Nitrification inhibition potential

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Introduction

Nitrogen is the most precious nutrient for crop growth and yield. Nitrification is a major pathway for nitrogen loss both in agricultural and natural system [1–3]. Nitrification results in transformation of the relatively immobile ammonium nitrogen (NH_4^+-N) to highly mobile nitrate (NO_3^--N) which promotes N losses through leaching of NO_3^--N as well as gaseous N emission caused potentially cascade of environmental and health problems [4–6]. Nitrous oxide (N_2O) is one of the major biogenic green house gases contributing to global warming, produced primary from denitrification processes in agricultural system [7]. Nitrification acts as a key process in determining fertilizer use efficiency by crops as well as nitrogen losses from soils. Arrest of N-loss in the crop fields can help in increasing N utilization. It is possible with the use of nitrification inhibitors either natural or synthetic.

Nitrifies activity increased by the availability of NH_4^+-N in soils either from fertilizer or released by the mineralization of soil organic matter [8]. The dicyandiamide (DCD) nitrification inhibitor improved the N fertilizer use efficiency and decreased NO_3^--N losses in citrus [9]. So, it helped to minimize the economic and environmental risks. DCD and neem cake were evaluated by for N inhibiting efficiency of prilled urea-derived NH_4^+-N in wheat [10]. It also reported that DCD was better than neem cake. The nitrogen use efficiency was 29 % in uncoated fertilizer and 46 % in polyolefin coated fertilizer with DCD was applied as a slow release fertilizers; especially coated urea with DCD significantly increase the N use efficiency in tea [11]. DCD was able to inhibit the nitrification process during the decomposition of crop residues [12].

Many tropical grasses having nitrifies activity, and recently *B. humidicola* attracted the attention of researcher

due to its inhibiting nitrification process, through root exudates [3]. It also reported that an effective nitrification inhibitor properties found in the root-exudates of the tropical forage grass *B. humidicola.* Seed germination of *B. humidicola* is a long process with very low germination rate, i.e. about 14 %. Its chemical composition called "brachialactone" contains cyclic diterpene with a unique 5-8-5-membered ring system and a γ-lactone ring. It contributed 60–90 % of the inhibitory activity released from the roots. Root exudates of *B. humidicola* concentrated and mixed with N fertilizers to minimize nitrification process and increase N use efficiency (NUE) in crops. The age and developmental stage of *B. humidicola* are very important in terms of root exudates quantity, and normally root exudates decreases with increasing plant age, especially after flowering which is coincidence with a reduction in microbial number [13].

The application of root extract did not change ammonium nitrogen content in soil up to 8th day [14]. Later on it observed that the ammonium oxidizing bacteria (AOB) populations and N_2O emission from the soil were significantly lower in the soil where *B. humidicola* has been grown compared to *B. decumbens* and *M. minutiflora*. Sorghum had significant BNIs capacity, releasing 20 allylthiourea units (ATU) g^{-1} root dry wt day^{-1}. BNIs compound release from roots is a physiologically active process, stimulated by the presence of NH_4^+ and growth stages of crops [15]. The percent nitrification inhibition of neem oil which was ranging from 4 to 30.9 % [16]. Use of BNIs compared to synthetic nitrification inhibitors i.e. as nitrapyrin, (DCD), and 3,4-dimethyl pyrazole phosphate showed many advantages in regards to NUE. The objective of the present study was to investigate the effect of root extracts of *B. humidicola* in relation to nitrification inhibition potential [17, 18].

Materials and Methods

Extraction Root Extracts from *B. humidicola*

Brachiaria humidicola was planted in pots having a mixture of soil and sand in the ratio of 4:1. Temperature was maintained at 20–26 °C during the growth period in greenhouse conditions. The plants of *B. humidicola* were uprooted after flowering stage from the pots and the roots were separated from the plants, cleaned and used for extraction. The macerated roots of *B. humidicola* were extracted with different solvents (70, 40 % ethyl alcohol, salt solution and phosphate buffer solution).

Incubation Study and Soil Analysis

A laboratory experiment was conducted for 22 days to determine the nitrification inhibition potential of root extracts. Ammonium sulphate was used as a source of nitrogen. Soil was collected from field of Indian Agricultural Research Institute (IARI), New Delhi, India. Soil samples were air-dried, ground and passed through a 2 mm sieve. Ten grams soil were mixed thoroughly with 10 g of quarts sand and placed in 250 ml conical flasks and treated with 1 ml solution containing 2 mg of nitrogen. Seven treatments were applied viz. control, four root extracts of *B. humidicola* called as biological nitrification inhibitors (BNIs) (i.e., 70 % ethyl alcohol, 40 % ethyl alcohol, phosphate buffer solution and 2 M KCl salt solution extracts) and two standard chemical nitrification inhibitors (NI) i.e. DCD and neem oil coating. Water holding capacity was maintained at field capacity during the course of study. Flasks were covered with aluminum foil caps having a central hole of 1 mm diameter and were incubated in incubator at 25 °C.

Soil samples collected at 14th and 22nd day after incubation and were extracted with 2 M KCl solution (soil:solution, 1:5). Nitrogen content in extract was determined by steam distillation [19]. The nitrification efficacy of extracts was determined by the technique materials and methods of Bundy and Brenner (1966) was used [20]. Soils samples analyzed for mineral nitrogen (ammonium N and nitrate N). The percent inhibition potential of the extracts as well as the standard inhibitors was computed.

Results and Discussion

Mineral: NH_4^+-N in Soil

In the study, NH_4^+-N in soil were significantly influenced by BNIs and NI. The NH_4^+-N in soil were highest in case of DCD 33.62 μg g^{-1} soil and in 70 % alcohol extract (26.19 μg g^{-1} soil) compared to rest of the treatments. Nitrification process was highly significant with time. The NH_4^+-N was higher (20.66 μg g^{-1} soil) at 14th day incubation period compared to 22nd day (11.91 μg g^{-1} soil) (Table 1). Nitrogen remained in the soil in inorganic form, NH_4^+-N, for a longer period at the spots where there was a growth of *B. humidicola* as compared to the adjacent spots having no growth of *B. humidicola.* The NH_4^+-N in soil was higher under DCD and 70 % alcohol extract, which clearly showed that the nitrification process was regulated more through DCD and 70 % alcohol extract due to suppressed growth of nitrifies bacterial population. The root exudates and soil extracts of *B. humidicola* suppressed AOB populations [14]. The synthetic nitrification inhibitors such as AT, nitrapyrin or DCD which are specific to the ammonia monooxygenase enzymatic pathway [21].When DCD was applied along with ammonium sulphate nitrate

Table 1 Effect of *Brachiaria humidicola* root extracts (BNIs) and nitrification inhibitors on NH_4^+-N, NO_3^--N and percent nitrification inhibition in soil

Treatments	NH_4^+-N (μg g^{-1} soil)			NO_3^--N (μg g^{-1} soil)			Nitrification inhibition (%)		
	Time (days)			Time (days)			Time (days)		
	14	22	Mean	14	22	Mean	14	22	Mean
T_1 (70 % alcohol extract)	30.35	22.02	26.19	5.85	18.76	12.31	131.37	89.12	110.25
T_2 (40 % alcohol extract)	14.53	8.68	11.61	10.01	24.4	17.21	118.18	70.86	94.52
T_3 (salt solution extract)	12.15	5.64	8.90	44.14	33.53	38.84	9.89	42.38	26.14
T_4 (buffer solution extract)	9.66	6.23	7.95	43.44	42.84	43.14	12.06	11.12	11.59
T_5 (neem oil coating)	20.06	6.23	13.15	24.22	31.36	27.79	73.09	48.34	60.72
T_6 (DCD)	44.35	22.89	33.62	27.34	30.59	28.97	63.12	50.88	57.00
T_7 (control)	13.55	11.69	12.62	40.30	45.27	42.79	45.30	34.74	40.02
Mean	20.66	11.91		27.09	32.39		64.71	49.63	
LSD ($P < 0.05$)	Treatment = 4.43			Treatment = 1.01			Treatment = 3.00		
	Time = 2.37			Time = 0.54			Time = 1.61		
	Treatment × time = 6.27			Treatment × time = 1.43			Treatment × time = 4.25		

(ASN), there were significantly higher levels of NH_4^+-N in the substrate than in the ASN treatment alone, for at least 100 days [9].

The NH_4^+-N in soil were distinctly higher at 14th days as compared to that of 22nd days (Fig. 1). The inhibitory effect of DCD and 70 % alcohol root extract was significant up to 14th day as compared to 22nd day. A complete suppression of nitrification by 20 U of BNIs per gram of soil which led to the retention of the added N [200 mg N as $(NH_4)_2SO_4$)] in NH_4^+-N form during a 55-day incubation. Nearly 40 % nitrification inhibition of soil was observed when applied BNIs released from sorghum to the soil reach at 10 ATU^{-1} soil [22].

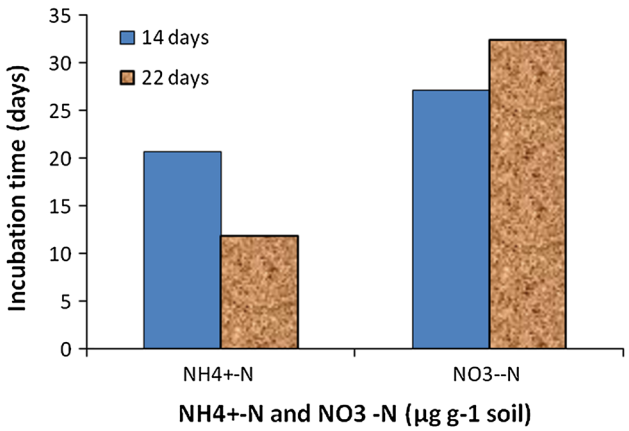

Fig. 1 Effect of incubation time on NH_4^+-N and NO_3^--N in soil

Mineral:NO_3^--N in Soil

On the contrary, NO_3^--N also measured in soil during incubation study, among the treatments highest NO_3^--N (43.14 μg g^{-1} soil) in soil was recorded in buffer solution extract. The NO_3^--N in soil increased with time, at 22nd day (21.69 μg g^{-1}) than 14th day (19.26 μg g^{-1}) (Table 1; Fig. 1). The nitrification process was inhibited by the root exudates of *B. humidicola.* The results support the hypothesis that *B. humidicola* suppressed nitrification and N_2O emissions through an inhibitory effect on the AOB populations [14]. Soil collected from field plots of 10 year-old high-BNIs genotypes of *B. humidicola,* showed a near total suppression (more than 90 %) of nitrification; most of the soil inorganic N remained in the NH_4^+ form after 30 days of incubation [17, 18]. Nitrification inhibitory activity in root tissues extracts of *B. humidicola* was significantly affected mineral NO_3^--N in soil [23].

Nitrification Inhibition

The maximum percent nitrification inhibition observed in 70 % alcohol extract (110.25 %) followed by 40 % alcohol (94.52 %), neem oil coating (60.72 %), DCD (57 %) (Table 1; Fig. 2). There was more percent nitrification inhibition in soil up to 14th day (64.71 %) compared to 22nd days (49.63 %). The synthetic nitrification inhibitor (DCD) suppress more than or equal 80 % on soil nitrification in the concentration range of 10–50 μg g^{-1} soil at 20 °C incubation temperature [22, 23]. The BNIs activity from root exudates was more effective in inhibiting nitrification process in soils than the standard nitrification inhibitor, nitrapyrin (at 4.5 ppm) during the period of the experiment [18]. The isolated compound brachialactone act as a nitrification inhibitor having γ-lactone ring. It reduced

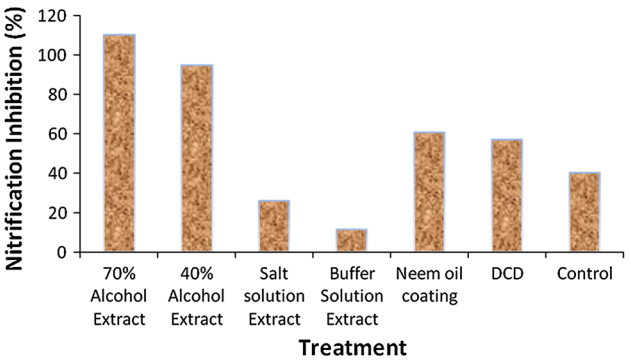

Fig. 2 *Brachiaria humidicola* root extracts (BNIs) and nitrification inhibitors effect on nitrification inhibition during incubation

the nitrification process 60–90 % and also the nitrification microbial population drastically [13].

Conclusion

It may be inferred from our study that the treatment of 70 % alcohol root extracts of *B. humidicola* showed higher suppression of nitrification process as compared to synthetic inhibitors like DCD. Therefore, it can be used as a natural nitrification inhibitor to check N-related problems, like ground water pollution, N_2O emission, and loss of N-inputs from agricultural fields. Further, BNIs were also effective in nitrification inhibition as compare to the synthetic nitrification inhibition DCD as well as the neem oil coating of fertilizers. These BNIs isolated as crude extracts of roots of *B. humidicola* can be used as a coating material on N fertilizers.

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