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Variation in Nitrification Inhibition Activity of Neem Leaves Collected from Different Locations of Lucknow (India)

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Authors' contributions

This work was carried out in collaboration by both the authors. Both the authors designed the study and author KA wrote the first draft of the manuscript. Author KA managed the literature searches, the experimental process and collected the data. Authors KA and AS analysed the data and drew inferences. Both the authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

This study was carried out to assess the variation in nitrification inhibition (NI) activity of neem leaves obtained from trees growing in same agroclimate. The study was accomplished in the year 2012, in Lucknow city of Uttar Pradesh State of India. Dried leaves of four neem trees growing at different locations within Lucknow were screened for their NI potential. Treatments comprised of 6 combinations of urea-N and inhibitors as urea with dried leaf powder of New Hyderabad (NH) location tree, urea with dried leaf powder of Gomti Nagar (GN) location tree, urea with dried leaf powder of Hazratganj (HG) location tree, urea with dried leaf powder of Indira Nagar (IN) location tree, urea alone (without any inhibitor) and control (without urea-N and without any inhibitor). Concentrations of NH_4^+-N and NO_3^--N present in soil were quantified on 1,3,7,14,21 and 28 day/s after treatment (DAT) by standard methods. Significant difference was observed in NI potential, as high concentration of NH_4^+-N was maintained during experimental period when dried leaf powder of NH location tree was used whereas low concentration of NH_4^+-N was obtained with the leaves of HG location tree. In the first week leaves of NH location tree, showed 90% NI which remained 56.98% till the third week, whereas it was least for HG by the third week, i.e., about 23%. Leaves obtained from NH location tree

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proved to be the best inhibitor for nitrification amongst all treatments. Further, growth response of seedlings of *Hordeum vulgare* (barley) was used as a biological check of soil nutritive value with the supplementation of dried leaf powder of four trees individually. NH location tree leaves treated soils were found to be the best with respect to growth parameters of barley. Study gives an indication for pre-screening of trees growing in different locations for better results at commercial levels.

Keywords: Neem; nitrification inhibitors; nitrogen loss; variation.

1. INTRODUCTION

Nitrogen (N) is an important nutrient required by plants for growth and development. Atmospheric N fixation by legumes, animal manures, soil organic matter (SOM) and N fertilizers constitutes the N pool in the soil. Urea, containing 46% nitrogen is an important nitrogen-fertilizer material used worldwide due to its cost effectiveness, desirable handling and storage characteristics. Nitrogen fertilizers are applied irrespective of soil conditions, climate, etc. but the fact is that about 70% of fertilizer in the field is lost due to nitrification and associated N losses, *viz*., leaching, ammonia volatilization and denitrification. Nitrification and other transformation processes not only ultimately result into N losses but also significantly contribute to environmental hazards. Excessive nitrification, denitrification and ammonia volatilization one way or the other adversely affect land, water and air, as acidification of soil due to increased levels of nitrate (NO_3^-), eutrophication of surface waters [1], contamination of groundwater with excessive nitrate [2] and gaseous emission through denitrification result in their accumulation above permissible levels leading to global warming. Nitrates leached in the soil reach groundwater where it gets accumulated over the permissible limit and causes groundwater contamination. The remedy for the above stated problems lie in the use of nitrification inhibitors which are chemicals that slow down, delay or restrict the nitrification process by retarding the metabolism of soil nitrifers as a result fertilizer N can be utilized by the plants before it is lost from the soil as nitrate [3]. Nitrification inhibitors stabilize the ammonium (NH_4^+) content, so that plants can easily uptake the N in this form [4]. There are many synthetic/chemical compounds as nitrification inhibitors available in market like, dicyandiamide [5], nitrapyrin [6] etc. but they are quite expensive and out of reach of a farmer. Teixeira et al. [7] assessed the effect of sources of N on wheat yields and concluded that efficiency of Entec® (ammonium sulfonitrate with nitrification inhibitor) was almost similar to ammonium sulphate and urea for most of the growth and yield parameters. Such reports suggest that these commercial nitrification inhibitors do not always work in tropical countries and in acids soils. Also the safety limits of these inhibitors for the mankind and environment is not very clear. On the other hand natural/herbal nitrification inhibitors are environment friendly, economically viable and easily available. Results of field experiments have shown comparable [8] or even better [9] performance of natural products when compared to synthetic nitrification inhibitors. In the same line, Neem parts as well as its products are known for its nitrification inhibition (NI) activity since long [10-13] but its genotypic variation has not been given attention. Neem being highly heterozygous in nature varies in content of its active principles widely. Meliacin (neem bitter- eg. nimbin, salanin, nimbidin, etc.) present in its parts are mainly responsible for nitrification inhibiting activity [10,12,14]. Variability in triterpenoids (nimbin and salanin) of neem trees located in the same agro-climatic zone has been suggested by other authors [15]. Similarly, neem oil obtained from different ecotypes showed differences in their activity as nitrification inhibitor [16]. Alsaadawi et al. [17] have reported genotypic differences in NI for sorghum root exudates and tissue extracts. Similarly, Subbarao et al. [18] have reported several high-and low-biological nitrification inhibition (BNI) genotypes within the *Brachiaria humidicola* species. Such genotypic variation with respect to biological nitrification inhibition potential has also been seen in the case of root exudates of rice [19]. Therefore, in view of this background, present study deals with the screening of neem trees growing at different locations of the same agroclimatic zone for variation in NI potential and growth of barley plants in treated soils.

2. MATERIALS AND METHODS

2.1 Experimental Work

Soil samples were collected from University garden at 30cm depth from an area that was not fertilized. One kg sieved soil (through 2mm sieve) was air-dried and placed in pots. The soil was tested for its physico-chemical properties using standard methods Table 1. Four trees of neem (*Azadirachta indica* A. Juss.; Family: Meliaceae) growing at different locations within Lucknow; New Hyderabad (NH), Gomti Nagar (GN), Hazratganj (HG) and Indira Nagar (IN) were screened for their NI potential Fig. 1.

Table 1. Properties of experimental soil (0-30cm)

Particulars	Value	Methods
pH (1 : 5:: Soil : Water)	7.5	Glass Electrode pH Meter [20]
Organic carbon (g kg^{-1})	3.4	Walkley and Black method [21]
NH_4^+-N (mg kg^{-1} soil)	43.4	Nessler method [22]
NO_3^--N (mg kg^{-1} soil)	3.8	Phenoldisulphonic method [23]

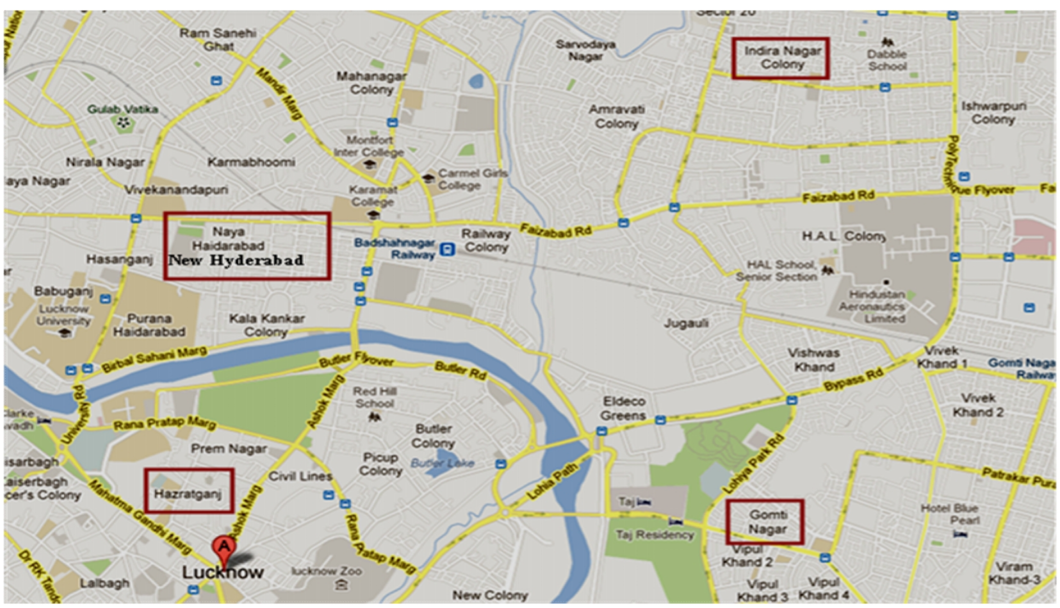

Fig. 1. Map of Lucknow city showing study sites. [Source: maps.google.co.in]

Leaves from mature neem trees almost of similar age growing at these four locations were collected and used as nitrification inhibitors. Treatments comprised of 6 combinations of N and inhibitors, *viz*., urea with dried leaf powder of tree of NH location, urea with dried leaf powder of tree of GN location, urea with dried leaf powder of tree of HG location, urea with dried leaf powder of tree of IN location, urea alone (without any inhibitor) and control (without N and without any inhibitor). Urea was applied at the rate 200mg N kg^{-1} soil. Dried neem leaf powder of different trees was used at the rate 30% (w/w) of urea applied. Concentrations of ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) present in soil were quantified on 1, 3, 7, 14, 21 and 28 day/s after treatment (DAT) by following the nessler method [22] and phenoldisulphonic method [23] respectively. The percent nitrification inhibition (%NI) was calculated as per Sahrawat et al. [24].

$$\%NI = \frac{[\text{Nitrification rate in control-nitrification rate in treatment}]}{\text{Nitrification rate in control}} \times 100$$

The soil water content was maintained at 60% of maximum water holding capacity and loss of soil moisture was replenished by adding distilled water. Soil was incubated at 25±2°C for 28 days.

To compare the inhibition activity (if any) of leaf powder of four trees growing at different locations on seed germination and plant growth, seeds of *Hordeum vulgare* (barley) were sown separately in pots containing 100g soil per pot with same treatments as mentioned above. Five seeds per pot were sown in three replicates and %germination, leaf length, number of leaves per plant and cumulative width (total width of tip, middle and basal region of a leaf) of leaves was recorded for 15 days.

2.2 Statistical Analysis

The design used was completely randomized design with three replications. Data were analyzed using analysis of variance (ANOVA) and the differences contrasted using a Duncan's multiple range test (DMRT). All statistical analyses; i.e., correlation, regression and DMRT were performed using the SPSS statistical software package.

3. RESULTS AND DISCUSSION

3.1 Variation in NI Activity of Neem Leaves Collected from Different Locations

The mineralization of nitrogen in urea was rapid from first week onwards and reached maximum at the 4th week of incubation. In all the treatments NH_4^+-N increased and NO_3^--N decreased till second week, later the condition reversed. The concentration of NH_4^+-N had a negative relationship with the corresponding NO_3^--N concentration in all treatments from 1DAT to 28 DAT [values of r being -0.72, -0.73, -0.71, -0.70, -0.74 and -0.50 for control, urea, NH, GN, HG and IN, respectively; Figs. 2(a-f). There was a good extent of variation observed among the leaves collected from different trees in their activity as nitrification inhibitor. Leaves collected from trees of NH and GN locations proved to be most effective as nitrification inhibitors as the concentration of NH_4^+-N was high and maintained for longer period of time i.e., 14 days. Though the leaves collected from trees of HG and IN locations also showed an increase in NH_4^+-N concentration till 7th day but 14th day onwards, the decline in NH_4^+-N concentration was significant Fig. 3. Percentage nitrification inhibition (%NI) was maximum in the NH location tree leaves followed by GN, IN and HG location tree leaves Fig. 4. With NH location tree leaves %NI was about 90% in first week and remained at 56.98% till the third week, whereas it was least, i.e., 23% with HG location tree leaves by the third week. NH maintained higher % NI till the fourth week, which was again maximum amongst four trees. Furthermore, nitrate-N was related to ammonium-N using regression methods. The growth parameters of barley were also related to ammonium-N concentration using regression methods. Earlier, also members of family Meliaceae have been reported to show promising results for their NI potentials. Toselli et al. [25] exploited derivatives of *Melia azedarach* L. and *Azadirachta indica* A. Juss. (melia leaves, melia fruits and neem cake) and showed their effect on increase in soil N availability, plant growth and ascertained their NI potential.

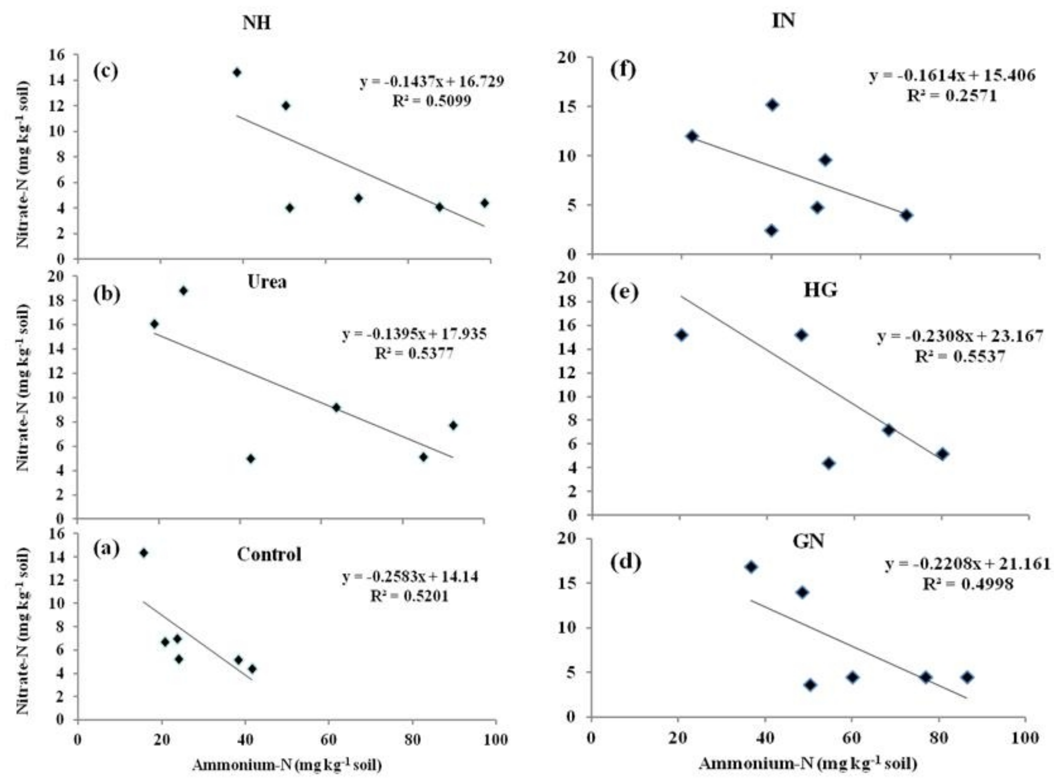

Fig. 2(a-f). Relationship between ammonium-N concentration and nitrate-N concentration from 1DAT to 28 DAT

Nitrification inhibition activity of neem leaves has been previously shown by several workers [12,26]. Neem bitter present in Neem has been reported to be responsible for nitrification inhibition [27,14]. The major constituents of bitters are nimbin, nimbinin, nimbidin, salannin, etc. present in different parts of neem tree but mainly concentrated in seeds. According to Santhi et al. [12] neem supplements reduce the population of Nitrifers (*Nitrosomonas, Nitrobacter* and *Nitrosococcus*). There was a difference in the NI activity of leaves of four trees used because, the location where the neem trees were growing could have influenced the production and concentration of allelochemicals in the neem trees. Activity of allelochemicals varies with temperature, photoperiod, water and soils, with its initial concentration, compound structure, with plant accessions, etc. [28]. Also other explanation for the same is that there exists an individual genetic difference among neem trees, and consequently the amount of bioactive compounds varies among trees growing even within same region [15]. Earlier reports also suggests that neem populations in India has a broad genetic base as assessed by different molecular markers [29,30]. Genotypic variation with respect to NI activity has also been reported in pasture *B. humidicola* [18] and rice [19]. A wide range of genetic variability for sorgoleone (inhibit *Nitrosomonas* activity) release-capacity in sorghum has been suggested [31]. Not only genetic difference results in variation of NI property but also, different extraction processes of neem oil influenced the potential [16].

In view of the above study and possible explanation for the same, it is recommended that comparative evaluation of trees from different locations be done. Leaves collected from trees growing in different locations which could also be distinct genotypes may be used in the formulation for effective inhibition after proper laboratory evaluation of their NI potential.

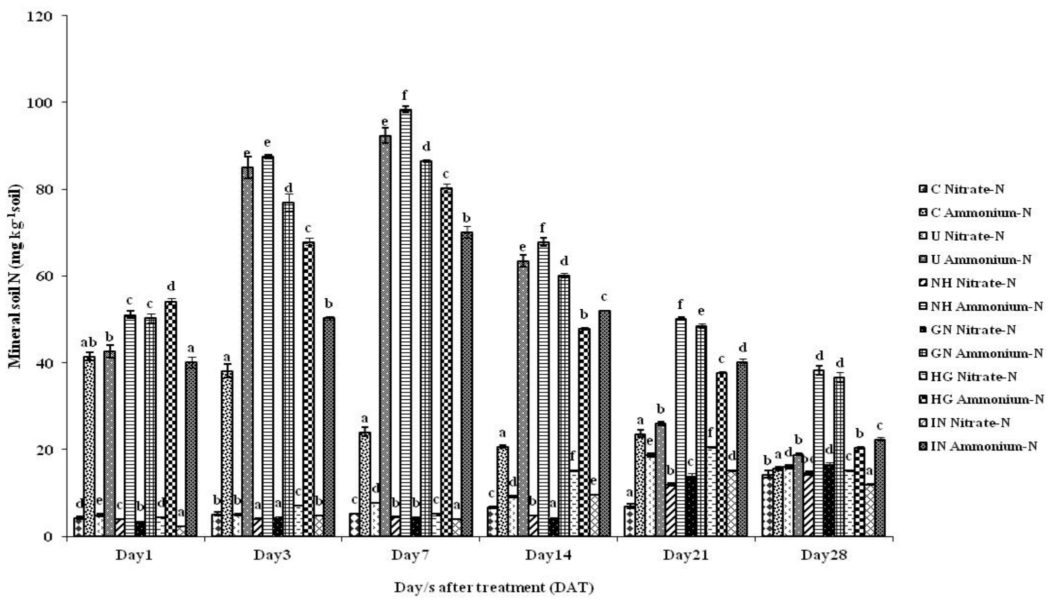

Fig. 3. Variation in transformation of urea-N in soil in different treatments

C: Control; U: Urea; NH: New Hyderabad; GN: Gomti Nagar; HG: Hazratganj; IN: Indira Nagar. Data are means ± SE of 3 replicates. Bars with different letters are significantly different at P ≤ .05, as determined by Duncan's multiple range test.

3.2 Growth of Plants in Treated Soils

Maximum % seed germination, i.e., 100% was obtained with leaves of NH and HG location trees followed by 80% germination with leaves of GN and IN location tree. Average number of leaves per plant and leaf length was relatively higher in plants in NH location tree leaves treated plants. Cumulative width of leaves was maximum in NH and HG followed by GN and urea treatment. Correlation was calculated between leaf length, number of leaves per plant and cumulative width of leaves with NH_4^+-N concentration of each treatment on 7 DAT when NH_4^+-N was maximum for all treatments Figs. 5(a-c).

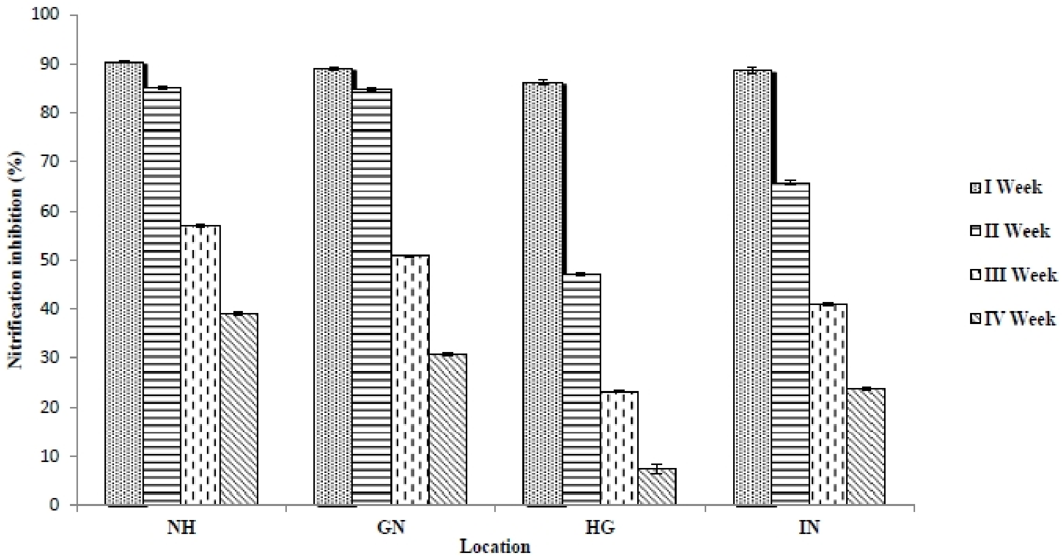

Fig. 4. Variation in percent nitrification inhibition amongst different trees.
NH: New Hyderabad; GN: Gomti Nagar; HG: Hazratganj; IN: Indira Nagar. Data are means ± SE of 3 replicates

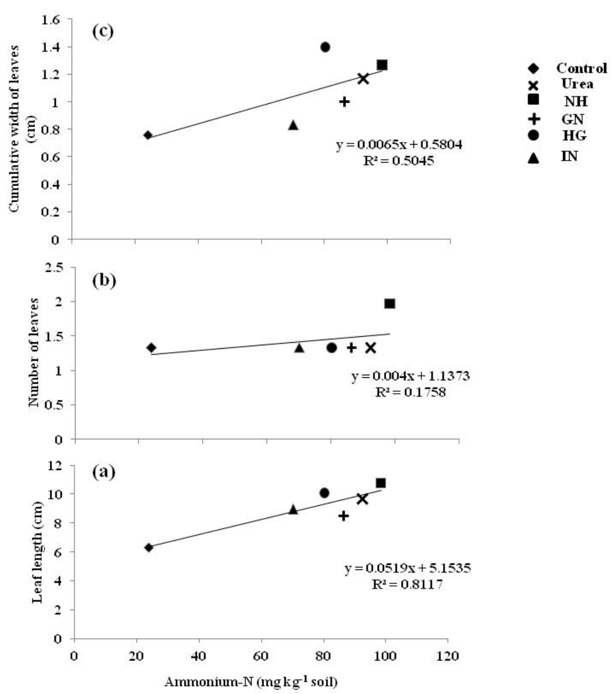

Fig. 5(a-c). Relationship between growth parameters and ammonium-N concentration on 7 DAT.(a) Leaf length; (b) Number of leaves per plant; (c) Cumulative width of leaves

A positive relation between growth parameters and NH_4^+-N concentration on 7 DAT with respect to treatments was observed. Plants were healthy and green in NH and GN location tree leaves treatment, whereas in HG and IN location trees leaves treated plants were poorly grown Fig. 6a. Leaves in HG showed necrosis, which is a common symptom of nitrogen deficiency Fig. 6b. This further confirms the variation in neem trees growing at different location within same agroclimatic zone as nitrification inhibitor.

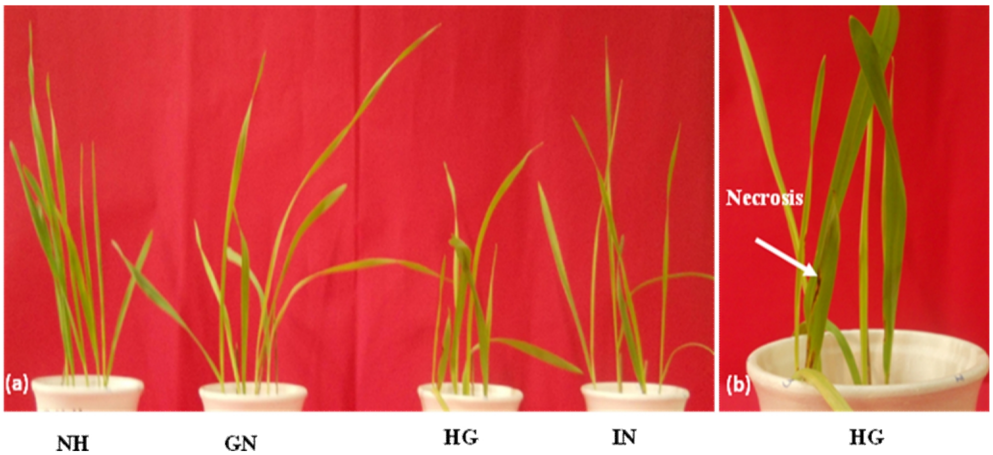

Fig. 6. (a) Growth of plants of *Hordeum vulgare* as an effect of leaf powder (inhibitor) of different trees.

NH: New Hyderabad; GN: Gomti Nagar; HG: Hazratganj; IN: Indira Nagar. (b) Necrosis in leaf powder of HG (Hazratganj) tree treated plant

4. CONCLUSION

In conclusion, with the known fact that neem leaves have potential to slow down nitrification, present analyses showed that there exists a difference in nitrification inhibition activity of neem leaves collected from trees growing at different locations. Also, a difference in growth of barley plants was observed in treated soils. Therefore, the screening of trees from different locations which may be distinct genotypes be done for maximum effectiveness.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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