Reducing options of ammonia volatilization and improving Nitrogen use efficiency via organic and inorganic amendments in wheat (*Triticum aestivum* L.)

Pooja L Ramalingappa [1], **Manoj Shrivastava** [1], **Shiva Dhar** [2], **Kalikinkar Bandyopadhyay** [3], **Shiv Prasad** [1], **Sapna Langyan** [4], **Ritu Tomer** [1], **Ashish Khandelwal** [1], **Sibananda Darjee** [1], **Renu Singh** [Corresp. 1]

[1] Division of Environment Science, ICAR-Indian Agricultural Reserach Institute, Delhi, India

[2] Division of Agronomy, ICAR-Indian Agricultural Reserach Institute, Delhi, India

[3] Division of Physics, ICAR-Indian Agricultural Reserach Institute, Delhi, India

[4] Division of Germplasm Evaluation, ICAR- National Bureau of Plant Genetic Resources, Delhi, India

Corresponding Author: Renu Singh
Email address: renu_icar@yahoo.com

Background. This study focuses to investigate the effect of organic and inorganic supplements on the reduction of ammonia (NH_3) volatilization, improvement in Nitrogen Use Efficiency (NUE), and wheat yield. **Methods.** A field experiment was conducted following Randomized Block Design with 10 treatments *i.e.,* T_1-without nitrogen (control), T_2-Recommended Dose of Nitrogen (RDN), T_3-(N-(n-butyl) thiophosphoric triamide (NBPT @ 0.5% w/w of RDN), T_4-Hydroquinone (HQ @ 0.3% w/w of RDN), T_5-Calcium carbide (CaC_2 @ 1% w/w of RDN), T_6-Vesicular Arbuscular Mycorrhiza (VAM @ 10 kg ha^{-1}), T_7-(Azotobacter @ 50 g kg^{-1} seeds), T_8-(Garlic powder @ 0.8% w/w of RDN), T_9-(Linseed oil @ 0.06% w/w of RDN), T_{10}-(Pongamia oil @ 0.06% w/w of RDN). **Results.** The highest NH_3 volatilization losses were observed in T_2 of about 20.4 kg ha^{-1} per season. Significant reduction in NH_3 volatilization losses was observed in T_3 by 40%, T_4 by 27%, and T_8 by 17% when compared to control treatment. Soil urease activity was found to be decreased in plot receiving amendments, T_3, T_4, and T_5. The highest grain yield was observed in the T_7 treated plot with 5.09 t ha$^{-1,}$ and straw yield of 9.44 t ha^{-1} in T_4. **Conclusion.** The shifting towards inorganic and organic amendments is a feasible option to reduce NH_3 volatilization from wheat cultivation and improves NUE.

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[1]Division of Environment Science, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India

[2]Division of Agronomy, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India

[3]Division of Agricultural Physics, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India

[4]Division of Germplasm Evaluation, ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India

Corresponding Author:
Renu Singh[1]
Division of Environment Science, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India
Email address: renu_icar@yahoo.com

Abstract

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Methods. A field experiment was conducted following Randomized Block Design with 10 treatments *i.e.,* T_1-without nitrogen (control), T_2-Recommended Dose of Nitrogen (RDN), T_3-(N-(n-butyl) thiophosphoric triamide (NBPT @ 0.5% w/w of RDN), T_4-Hydroquinone (HQ @ 0.3% w/w of RDN), T_5-Calcium carbide (CaC_2 @ 1% w/w of RDN), T_6-Vesicular Arbuscular Mycorrhiza (VAM @ 10 kg ha^{-1}), T_7-(Azotobacter @ 50 g kg^{-1} seeds), T_8-(Garlic powder @ 0.8% w/w of RDN), T_9-(Linseed oil @ 0.06% w/w of RDN), T_{10}-(Pongamia oil @ 0.06% w/w of RDN).

Results. The highest NH_3 volatilization losses were observed in T_2 of about 20.4 kg ha^{-1} per season. Significant reduction in NH_3 volatilization losses was observed in T_3 by 40%, T_4 by 27%, and T_8 by 17% when compared to control treatment. Soil urease activity was found to be decreased in plot receiving amendments, T_3, T_4, and T_5. The highest grain yield was observed in the T_7 treated plot with 5.09 t ha^{-1}, and straw yield of 9.44 t ha^{-1} in T_4.

Conclusion. The shifting towards inorganic and organic amendments is a feasible option to reduce NH_3 volatilization from wheat cultivation and improves NUE.

Introduction

Nitrogen (N) in the atmosphere is the principal source of all soil nitrogen. It naturally enters the soil through dead animal and plant residues, biological nitrogen fixation, and chemical N fertilizer applications. Nitrogen fertilizers have become an essential input in agriculture to increase crop yield and enhance food quality (*Leghari et al., 2016*). Food grain crops account for more than 69% of India's total N fertilizer intake, where wheat alone has its share of 24%. In the crop year 2020-21, India's wheat production has increased from 109 Mt to 118 Mt and to meet the demand of growing population wheat yield has to be increased by 1.5% per year (*Grain and Feed Annual report, 2021*). Ammonia volatilization is a significant cause of nitrogen depletion in agricultural soil worldwide, contributing to low fertilizer N usage, production, and indirect nitrous oxide (N_2O) emissions. Globally, the average NH_3

volatilization risks range from 0.9 to 64 % of the applied N (a mean of 17.6 %) (*Pan et al., 2016*). Ammonia volatilization into the atmosphere negatively affects agriculture, ecosystems, and human health. Further, it also increases the loss of nitrogen for plant growth, thus increasing the cost of cultivation (*Brink & Van Grinsven, 2011*). Secondary particulate matter (PM10) is formed when NH_3 reacts with other air contaminants such as sulfuric acid and nitric acid. It flies long distances and remains in the air for several days, and causes respiratory diseases in humans (*Bittman et al., 2014*).

A study conducted by Cao et al. (*2013*) found that the significant loss of N from applied fertilizer was through NH_3 volatilization, which was estimated to be 10.0-19.5% of total N loss, further concluded that NH_3 volatilization might be the dominant pathway of N loss. Ammonia volatilization favorably occurs in organic N fertilizer like urea and organic manure. Ammonia emission is undesirable as it takes out N from soil/plant system and release into the atmosphere which will contribute to acid rain. Volatilized NH_3 is deposited back to earth's surface mainly through two processes they are i) Wet deposition through precipitation, ii) Dry deposition when combined with particulate matter. This has serious impact on environment as it causes acidification of soil, water bodies and eutrophication of natural ecosystem. Also acts as indirect source of N_2O which is a potential greenhouse gas. In certain cases it also has direct toxicity on plants under high concentration in combination with low temperature. There are many other ways to control N loss, like avoiding the application of urea under high-risk conditions, deep placement, and use of controlled-release N-fertilizers. But the more effective way to control is by using organic and inorganic amendments; some of them are chemical inhibitors like N-(n-butyl) thiophosphoric triamide (NBPT), cyclohexyl phosphoric triamide (CHPT), Ammonium thiosulphate (ATS), Hydroquinone (HQ), Calcium carbide (CaC_2) etc. Among these NBPT has been used globally, being the most effective in a market that has expanded at a rate of 16 % per year over the last ten years. NBPT-treated urea reduces NH_3 loss by around 53% (*Cantarella et al., 2018*). Application of 12 kg N ha^{-1} HQ on an Alluvial soil, in conjunction with 120 kg urea-N ha^{-1}, decreased N_2O emission by 5% in rice and 7% in wheat systems when compared to the crops grown solely in the presence of 120 kg N ha^{-1} urea (*Modolo et al., 2018*).The slow-N-release coating technology is also a suitable method for consistent N supply to the plants and reducing loss and contamination effects. Still, there are many organic and inorganic amendments that we can explore to utilize to minimize the NH_3

volatilization losses and enhance the productivity and NUE of the wheat crop. The application of N inhibitors along with urea has increased the average grain yield by 6.8% (*Školníková et al., 2022*). Nitrification and urease inhibitors are being suggested as a way of decreasing N losses and thus increasing the efficiency of crop nitrogen usage (*Abalos et al. 2014*). Different mechanisms are involved in reduction of NH_3 volatilization in that NBPT retards the activity of urease enzyme by competitive inhibition (*Fan et al., 2018*). Hydroquinone and Calcium carbide are also effective in reducing the urease activity. Application of HQ to the soil not only inhibited urease enzyme activity but also inhibited or enhanced the activity of other enzymes like polyphenol oxidase, dehydrogenase, protease and phosphatase (*Wołejko et al., 2020*). The CaC_2 has negative impact on ammonia oxidizing bacteria which are present in the soil, also reduces the activity of dehydrogenase and nitrate reductase enzyme there by reducing the N losses (*Mahmood et al., 2014*). Use of pongamia oil (karanjin) as nitrogen loss inhibitor works mainly by inhibiting the nitrification by reducing the activity of nitrosomonas without affecting the activity of nitrobacter. The treatment with garlic extract which is rich in compound thiosulfinate works on mechanism of competitive inhibition of urease enzyme as it contains organosulphur group (-S(O)-S) which is similar to urea which helps in inhibiting hydrolysis process (*Mathialagan et al., 2017*).

The world's population will surpass 9.7 billion by 2050, posing a significant obstacle to achieving food sustainability. The projected increase in the world's population demands at least a 70% increase in agriculture production in developed countries and 100% in developing countries (*Mahmud et al., 2021*). Food security of the country and N-based global warming and environmental degradation are interlinked (*Bilal & Aziz, 2022*). The use of urease inhibitors in agricultural activities has already been investigated as one of the best methods for ensuring adequate nutritional security (*Modolo et al., 2018*). N-(n-butyl) thiophosphoric triamide treated urea lowers NH_3 loss by approximately 53%. The yield benefit from NBPT application is 6.0 % and ranges from 0.8 to 10.2 % depending on crop types (*Cantarella et al., 2018*). The effectiveness of NBPT in reducing NH_3 loss is well known, but there is still room for progress to increase the amount of inhibition and hence the retention of NBPT-treated urea in the field. However, these inorganic amendments are not economically viable, and most of them restricted their usage in the research areas due to high cost. Upadhyay, Tewari & Patra, (*2011*) investigated that these chemicals are harmful and inhibit the growth of specific beneficial soil microorganisms that indirectly affects crop growth and development; thereby, the following best option stands are organic amendments.

Organic amendments are eco-friendly, economically viable, and, most importantly, biodegradable. Therefore, they act as promising N inhibitors. There are many plant-derived inhibitors like neem (*Azadirachta indica*) oil, pongamia (*Pongamia glabra*) oil, linseed (*Linum usitatissimum*) oil, garlic extract, and mint (*Mentha spicata*) as N amendments in inhibiting N losses. Thiosulfinates (TS) present in fresh garlic extract acts a bio-inhibitor of urease enzyme activity can be used as potential urease inhibitor in agriculture. Thiosulfinates has potential to inhibit urease enzyme in 200 minutes after application (*Ramli et al., 2014*). Application of coated urea fertilizers such as neem coated urea and pine oleoresin coated urea in a vertisol reduced the NH_3 volatilization by 27.5% and 41.1%, respectively (*Jadon et al., 2018*). But these organic amendments lack commercialization and efficient utilization. So this study concentrates mainly on stressing the importance of these organic amendments in comparison with inorganic amendments and decreases the contamination of soil and environment in parallel with increasing NUE.

With this interest, a study on “Reducing ammonia volatilization using organic and inorganic amendments in Wheat (*Triticum aestivum* L.)” was studied with two objectives. First objective is to study the effect of organic and inorganic amendments on ammonia volatilization and second one is to evaluate the effect of organic and inorganic amendments on the NUE and productivity of wheat. We hypothesize that the organic and inorganic amendments have the potential to reduce NH_3 volatilization, increase the NUE and yield of wheat.

Materials & Methods

Details of the experimental site

A field study was taken at the research farm of the Indian Agricultural Research Institute, New Delhi, located at 280º 40 'N and 770º 12' E, at an altitude of 228.16 m above mean sea level (sub-tropical and semi-arid region), during November 2020 to April 2021. The Southwest monsoon contributed about 80% of this rainfall, with an average of 650 mm annually. The pH of the soil was 8 (±0.1), with a sandy clay loam texture. The initial soil available N was low (157±0.5 kg ha^{-1}), medium level of available P (13.5±0.2 kg ha^{-1}) and available K (196±0.8 kg ha^{-1}).

Experimental design and management

The experiment was conducted in a randomized block design (RBD) with 3 replications, and each plot area was 12 m^2 (4 m×3 m). In all treatments recommended dose of nitrogen (RDN) was applied except T_1 (control), where no RDN was used. The research was carried out with growing wheat variety (HD 2967) with 10 treatments in respective plot namely T_1 (control),T_2 (RDN @ 150 kg ha^{-1}), T_3 (RDN + NBPT (N-(n-butyl) thiophosphoric triamide) @ 0.5% w/w RDN), T_4 (RDN +HQ (Hydroquinone) @ 0.3% w/w of RDN), T_5 (RDN + Calcium Carbide @ 1% w/w of RDN), T_6 (RDN + VAM (Vesicular Arbuscular Mycorrhiza @ 10 kg ha^{-1}), T_7 (RDN + Azotobacter @ 50 g kg^{-1} seeds) T_8 (RDN + Garlic powder @ 0.8% w/w of RDN), T_9 (RDN + Linseed oil @ 0.06% w/w of RDN), T_{10} (RDN + Pongamia oil @ 0.06% w/w of RDN). Each dose was finalized based on the current usage of these amendments by firms, which were applied in 2 splits (One at the time of sowing and the second split application 30 days after sowing). To study the effect of organic and inorganic amendments together three inorganic amendment, two biofertilizers and three organic amendments were chosen based on popularity and review of literatures. N-(n-butyl) thiophosphoric triamide (*Zanin et al., 2015*), Hydroquinone (*Modolo et al.,2018*), Calcium Carbide(*Sakariyawo et al., 2020*), Garlic powder (*Ramli et al., 2014*), Linseed oil, and Pongamia oil (*Majumdar, 2008*) were mixed with Urea (RDN) as per dosage. Wheat seeds were treated with azotobater (as per ICAR RABI Agro-Advisory For Farmers) and used for sowing in T_7. Vesicular Arbuscular Mycorrhiza (as per ICAR RABI Agro-Advisory For Farmers) was applied directly into the soil during sowing with a RDN. The recommended dose of fertilizers (RDF) for wheat was 150:60:40 kg ha^{-1} N: P_2O_5:K_2O. Urea, single super phosphate (SSP), and muriate of potash (MOP) were used as a source of N, P_2O_5, and K_2O, respectively.

Method of Soil sample collection and analysis

Fresh soil samples were obtained from the 0-15 cm layer of soil at three separate sites from each treatment using an 8 cm tube auger. Three soil samples were obtained from each treatment during tillering, flowering, grain filling, and physiological maturity of the crop. The fresh soil samples were 30 in number and air-dried for 7 days, sieved through a 2 mm screen, mixed, and placed in plastic bags for further analysis

Collection and analysis of NH_3

The ammonia volatilization was monitored after fertilizer application up to 10 days by using Forced air draft system method (*Bhaskar et al. 2022; Stumpe et al. 1984; Bremner, 1965*). The closed-chambers measuring 20 cm × 20 cm × 50 cm size made of 6 mm acrylic sheets would be placed in field. The volatilized NH_3 gas from soil surface under different treatments will be collected in 2 % Boric acid solution containing mixed indicator (methyl red and bromocresol green). The air inside the chamber will be collected into boric acid traps using vacuum pump having a flow rate of 3 L min^{-1}. The boric acid traps will be changed after every 24 hr. The volatilized NH_3 can be determined by the titration of boric acid solution with 0.02 N sulphuric acid, further calculated using formula.

Total Nitrogen and NH_4^+-N and NO_3^--N analysis

Total nitrogen content in soil was determined by the Kjeldahl method (*Kjeldahl, 1883*) during the initial and after harvesting of crop growth. The NH_4^+-N and NO_3^- -N were analyzed through Steam distillation method (*Keeney and Bremner, 1965*) during all 4 crop growth stages (Tillering, Flowering, Grain-filling, Physiological maturity). The extract was prepared by taking 10 g soils with 0.25 g activated charcoal and 50 mL KCl Solution and kept for shaking (30 min) then filtered with Whatman filter paper 1.From the same extract 10 mL of KCl extract was taken in two different distillation flask and 100 mL distilled water was added to each flask. 1g Devardas alloy was added in case of NH_4^+-N estimation and forNO_3^--N estimation 1.0 g magnesium oxide (MgO) was added and distilled separately and captured in 20 mL 2% Boric acid and titrated against 0.02 N sulfuric acid. Further calculation was done. Available Phosphorus was analyzed using Olsen method of estimation (*Olsen et al., 1954*). Available P from soil sample was extracted using 0.5 N $NaHCO_3$ solution buffer alt pH 8.5 then P in the extract was measured by ascorbic acid method using spectrophotometer. Available potassium in the soil were measured using ammonium acetate method (*Hanway & Heidel, 1952*), where available K was extracted by shaking with neutral normal ammonium acetate for 5 min the K is determined using flame photometer. Soil Organic Carbon was measured using Walkley and Black's rapid titration method (*Walkley & Black, 1934*).

Analysis of Nitrous oxide (N_2O) and others significant parameters

Nitrous oxide flux was analyzed using the closed chamber method (*Herr et al., 2020*). In this method, dark PVC boxes were installed, and samples were drawn every 24 hrs in the morning using syringes and evacuated into plastic vials, and analyzed chromatographically. Denitrification losses were estimated by denitrification enzyme assay as described by Smith & Tiedje (*1979*).

Soil urease activity was also analyzed at the flowering stage, calorimetrically by Bremner & Dauglas (*1971*) method. Normalized Difference Vegetation Index (NDVI) was measured using Green seeker (handheld crop sensor by Trimble) at 50% flowering stage. IRGA (LI-COR Model LI-6400X7 portable photosynthetic system) was used to measure the photosynthetic rate and stomatal conductance. Soil Microbial Biomass Carbon (MBC) and Soil Microbial Biomass Nitrogen (MBN) were determined by the chloroform fumigation–extraction method described by Vance et al. (*1987*) and Brookes et al. (*1985*), respectively. The N content in grains and straw was also measured using the Kjeldahl method (*Kjeldahl, 1883*). After harvesting of the crop, yield attributes were calculated from each plot.

Calculation of Nitrogen Use Efficiency in wheat

The Nitrogen Use Efficiency can be defined as the ratio of outputs to inputs (i.e. NUE = N yield/N input).

Agronomic Efficiency of N (AEN) (kg ha^{-1}) = $\frac{GYF - GYN}{AFN}$..........Eq. 1

Production Efficiency of N (PEN) (kg kg^{-1}) = $\frac{GYF - GYN}{TUN - CUN}$..........Eq. 2

Apparent N Recovery (ANR) (%) = $\frac{TUN - CUN}{AFN} \times 100$.........Eq. 3

TUN = Total N uptake from the fertilized plot (kg ha^{-1})

CUN = Total N uptake from unfertilized / control plot (kg ha^{-1})

AFN = Amount of applied fertilizer N (kg ha^{-1})

GYF = Grain yield in the fertilized plot (kg ha^{-1})

GYN= Grain yield in unfertilized/control plot (kg ha^{-1})

2.7 Statistical analysis

The measurements obtained from the experimental work were analyzed by using OPSTAT,1 Software (O.P. Sheoran, Hisar, India) and calculated ANOVA, and means separated using Duncan Multiple Range Test (DMRT) at α=0.05. The data for each variable was evaluated using variance protocol analysis for a Randomized Block design, which was checked using the “F” test for statistical significance (*Gomez & Gomez, 1984*). For each parameter, the

Standard Error of Means (SEm) and Critical Difference (CD) were calculated at a 5% level of significance.

Results

NH_3 volatilization losses during the wheat growth period

Additions of both organic and inorganic amendments have effectively controlled the ammonia volatilization losses. Ammonia losses were measured during the initial 5 days after applying fertilizer (*Bhaskar et al., 2022*), which was applied in 2 splits (0 DAS and 30 DAS). The temporal graph shown in figure 1 depicts two peaks of NH_3 flux in the entire cropping season. Significant losses through NH_3 volatilization were observed in the initial 5-6 days after fertilizer application. The highest volatilization losses were observed in T_2 of about 20 kg ha^{-1} $season^{-1}$ and the lowest was found to be in T_1 i.e. 6.4 kg ha^{-1} $season^{-1}$. T_3 performed well in reducing the losses by 40% when compared to the NH_3 losses in T_2. T_3, T_4 and T_8 showed the lowest emissions of NH_3 among all the treatments by losing 12.4, 14.6, 16.6 kg ha^{-1} $season^{-1}$, respectively to the atmosphere (Figure 2). Among plant-based amendments, T_8 with garlic powder treated urea showed better results by reducing NH_3 volatilization losses by 34% compared to only RDF treated urea (T_2). Duncan Multiple Range Test revealed that T_3 (NBPT) and T_4 (HQ) were effective in reducing the NH_3 losses.T_5 (CaC_2) and T_8 (Garlic powder) showed no significant difference among their means i.e had similar effect in reducing NH_3 losses next to NBPT. Other treatments were not that effective in reducing the NH_3 losses.

Initial and final soil nutrient status

The total nitrogen content and available nitrogen content in the soil, when analyzed after harvesting of the crop, were found to be highest in plot T_7, i.e., Azotobacter treated plot (0.041% and 195 kg ha^{-1}, respectively), followed by T_6, i.e., VAM treated plot (0.039% and 187 kg ha^{-1}, respectively) Available N content for different treatments are given in table 1. The highest SOC was observed in T_7, treated with azotobacter, i.e., 0.63%. It has significantly increased the SOC content when compared to the initial SOC (0.42%) before sowing of the wheat crop as shown in figure 3. The highest levels of P were observed in T_6 treated with VAM, i.e., 30.4 kg ha^{-1}, which is more than double the amount of initial value of available P as shown in table 1. The initial level of available K was 196 kg ha^{-1}, while in the final analysis, it was increased up to 229 kg ha^{-1} in T_{10}, as depicted in table 1.

Ammonical N (NH_4^+-N) and Nitrate (NO_3^-—N) in soil

The available nitrogen forms, majorly NH^{4+}-N and NO_3^--N in soil, are found to be decreasing from tillering stage to the grain filling stage (Figure 4.). The mean quantity of NH_4^+-N in soil at various growth stages (tillering, flowering, grain filling stage, physiological maturity stage) were found to be 78.6 kg ha^{-1} , 65.6 kg ha^{-1} , 57.9 kg ha^{-1} , 53.5 kg ha^{-1} respectively. The mean of NO_3^- -N in soil at all four growth stages were found to be 70 kg ha^{-1} , 63.4 kg ha^{-1} , 56.5 kg ha^{-1} , 51.1 kg ha^{-1} respectively. During the tillering stage, the highest NH_4^+-N (108 kg ha^{-1}) was observed in T_3, i.e., treatment with NBPT amended urea, and the highest NO_3^--N (58.5 kg ha^{-1}) was found in RDN treated plot (T_2). In all the stages of plant growth T_3 showed significantly higher level of NH_4^+-N which was 47% higher than T_2. Duncan Multiple Range Test also showed that the mean of treatments T_4, T_7 and T_8 also significantly at par for available NH_4^+-N content in soil and in case of NO_3^-—N availability, mean of treatments T_4, T_7 and T_5 also showed significant difference during all the stages of the wheat crop growth.

Nitrous oxide flux and denitrification losses from soil

Nitrous oxide emission is directly related to the amount of N fertilizer available in the soil. The highest cumulative N_2O flux was observed in T_2 (RDF) plots with 6.52 kg N_2O-N ha^{-1} $season^{-1}$. There were no significant differences between T_3, T_4, and T_5 in the case of N_2O emission reduction (Figure 3). But all of them were significant when compared with T_2. Among plant-based inhibitor treatments, T_8, having urea amended with garlic powder, showed effective results. The highest denitrification losses were observed in T_2 (3.66 kg-N ha^{-1}) (Figure 5). N-(n-butyl) thiophosphoric triamide treated urea was comparatively effective by reducing the denitrification losses by 35 %.

Soil microbial biomass carbon and nitrogen in soil

Soil biomass carbon, and soil biomass nitrogen (found within living organisms like fungi and bacteria) were observed at the flowering stage of the crop. The highest MBN content was reported in T_7, i.e., Azotobacter treated plot with 37 mg kg^{-1}, followed by T_6, having VAM treated plot. Highest MBC (136 mg kg^{-1}) was observed in T_7, which is Azotobacter treated plot, and it was 63.9% more than that of T_2 (Figure 6).

Soil urease enzyme activity in soil

As observed in figure 7, under the flowering stage in $T_{2,}$ i.e., only RDN treated plot showed the highest urease activity (18.9 mg urea g^{-1} soil h^{-1}), and the lowest urease activity (7.71 mg urea g^{-1} soil h^{-1}) was observed in T_3 treatment containing NBPT which was indicated by the effectiveness of urease inhibitor.

Physiological parameters of wheat crop

Addition of organic and inorganic amendments to the soil has significantly influenced the physiological parameters (photosynthesis rate. The range of photosynthesis rate was varied from 15.7 μ mol m^{-2} s^{-1} to 23.8 μ mol m^{-2} s^{-1} which was recorded during 50% flowering stage. The highest photosynthetic rate was observed in T_7 with 23.8 μ mol m^{-2} s^{-1} followed by $T_{3,}$ T_6 (23.3 μ mol m^{-2} s^{-1} and 22.9 μ mol m^{-2} s^{-1}). The range of chlorophyll content was varied from 0.66 to 0.77 which was recorded during 50% flowering stage. The highest chlorophyll content was observed in both T_4 and T_7 with 0.77 followed by T_6 and T_{10} showing 0.76. The highest LAI was observed in T_7 with 4.59 followed by T_3 and T_8 which has recorded the same LAI i.e. 4.16. The least reading was recorded in treatment T_1 i.e. 1.85. The highest stomatal conductance was observed in T_7 with 0.48 m mol m^{-2} s^{-1} followed by T_3, T_8 (0.47 m mol m^{-2} s^{-1} and 0.43 m mol m^{-2} s^{-1}), respectively.

Nitrogen uptake by wheat

The data about the nitrogen content analyzed in grain and straw samples of wheat after harvest depicted the positive effect of both organic and inorganic amendments. The azotobacter treated plot (T_7) showed 38.7% higher nitrogen content in grains when compared to T_2 treatments, as shown in table 1. The highest nitrogen content in straw was observed in T_3 (NBPT), and it was almost double the straw nitrogen content of T_1 (control) plot.

Wheat yield and nitrogen use efficiency

The data obtained in grain and straw yield showed significant improvement in yields due to the addition of organic and inorganic amendments. The highest grain yield was observed in $T_{3,}$ i.e., treatment with NBPT amended urea with 5.09 t $ha^{-1,}$ and straw yield was in T_4, i.e., treatment with hydroquinone amended urea (Table 2) but in case of straw yield all the treatment means were not significantly different at p=0.05 . The other yield attributes like biological yield, number of tillers per m^{-2}, number of spikes per m^{-2}, number of grains per spike, and harvest index also were not significantly influenced by amendments addition (p=0.05). The test weight of the wheat grain of treatment T_8 (39.8 g) was significant among all the treatments. .

As nitrogen content in the grains has increased on amending urea with organic and inorganic amendments, NUE was calculated in terms of AEN, PEN, and ANR using Equations 2, 3, and 4. The highest AEN was found in T_3 with 12.9 kg $ha^{-1,}$ and the lowest was in $T_{2,}$ 9.05 kg ha^{-1} (Table 1). The highest PEN was observed in T_2 with 25.8 kg $kg^{-1,}$ and the lowest was in $T_{7,}$ (15.4 kg kg^{-1}). The average value of PEN was 17.9 kg kg^{-1}. The highest ARN (81 %) was seen in $T_{7,}$ and the lowest was in T_2 (35 %). The mean value of ANR was observed to be 49.6%.

Discussion

Our major findings in case of NH_3 volatilization losses depicted that T_3 out performed compared to all other treatments because of competitive inhibition of urease enzyme activity by NBPT in the soil which has a major role in urea hydrolysis process where urea molecule is converted into ammonium ion. Next to NBPT, the other inorganic amendments significantly decreased the NH_3 volatilization losses. The allicin (thiosulfinate) naturally present in garlic is a bio- inhibitor of urease activity, as it contains an organosulfur functional group similar to that of the ureas. This might help allicin to decrease urease activity. This is in agreement with the research study conducted by Mathialagan et al. (*2017*). These amendments have practical implications in reducing the quantity of urea applied to the field there by reducing the N losses. Even though many studies are taken up in case of NBPT, still there is need to improve the duration of inhibition of urease activity, shelf life of urea fertilizer coated with NBPT and economic viability of these compounds. Agriculture alone contributes 80-90% NH_3 emissions globally mainly through volatilization from livestock and synthetic nitrogen fertilizer (*Xu et al., 2019*). It acts as pollutant influencing biosphere by formation of haze and soil acidification. N_2O which is potential greenhouse gas also of a great concern which contributes in global warming and affects human and environmental health. Li et al. (*2015*) reported that the Limus® (a new urease inhibitor consisting of 75% NBPT and 25% N-(n-propyl) thiophosphoric triamide (NPPT)), showed on an average 83% decrease in NH_3 losses during winter wheat season in China. Only the addition of NBPT can reduce NH_3 volatilization losses by 61% to 74%, as reported by Lasisi, Akinremi & Kumaragamage (*2019*). In his study on the efficiency of winter versus spring-applied urea-based fertilizers treated with urease and nitrification inhibitors. Affendi, Mansor & Samiri (*2019*) conducted a study by adding various chemical and natural urease inhibitors to reduce ammonia and nitrous oxide losses from soil. They reported similar findings in a combination of thiosulfate with urea. However, they found NBPT was more effective than the combined use of thiosulfate with urea to reduce NH_3 volatilization losses. Similar results were reported by Eduardo et al. (*2016*), he reported a decrease of 31.6% N, which used to be lost if not applied with thiosulfinates, and concluded it as an upcoming natural urease inhibitor.

The final analysis of TNC, available N, P and K reported increase in all these nutrient contents than the initial analysis which helps the next sown crop in nutrition. T_7 treated with azotobacter performed well in increasing N and K content in soil and T_6 treated with VAM in increasing P content in soil. Increase in N content of the soil could be due to the addition of these bio fertilizers as amendments that significantly increased residual N and reduced the N fertilizer application for the next crop, which will be sown in that plots. Vesicular Arbuscular Mycorrhiza improves the mobilization of P; hence uptake by wheat crop and P content in soil is increased. Addition of biofertilizers as a source of amendments in order to reduce the N losses has improved the SOC of the soil. The highest SOC was observed in T_7 which was treated with azotobacter i.e. 0.63%.It has doubled the SOC content when compared to initial SOC before sowing of wheat crop i.e. 0.42%. A similar effect of these bio fertilizers in increasing total nitrogen content was observed in a study on Bio-fertilizer affects structural dynamics, function, and network patterns of the sugarcane rhizospheric microbiota by Liu et al. (*2021*). Kader et al. (*2002*) also found similar results while working on the effect of azotobacter inoculants on wheat's yield and nitrogen uptake. This result is supported by Suri et al. (*2011*) while working on the influence of VAM and applied P on root colonization in wheat.

The available N forms majorly NH_4^+-N and NO_3^--N found to be decreasing from tillering stage to grain filling stage due to uptake and losses. During tillering stage the highest NH_4^+-N and NO_3^--N was observed in T_3 i.e. treatment with NBPT amended urea due to slow down of urease activity thereby enhancing the accumulation of NH_4^+ and NO_3^- ions and this increases the plant uptake. The urease activity was slowed down when NBPT amended urea was applied to soil, thus enhancing the accumulation of NH_4^+ ions, increasing the plant uptake of NH_4^+ and NO_3^- ions. During N mineralization from the applied N fertilizers, most of the N remains as NH_4^+ ions, then rapidly converting into NO_3^- due to the inhibitory effect of NBPT.

This also helps in reducing NO_3^- leaching. A similar result was found by Dhakar et al. (*2015*) while working on the impact of nitrification inhibitors and various nitrogen sources on soil nitrogen distribution in kinnow orchards.

As the availability of the NH_4^+ was initially low for the conversion into N_2O, the losses were low in the initial 7 days due to the application of these inhibitors. The application of urease inhibitors as amendments and urea regulate the concentration of NH_4^+ and NO_3^- in the soil, thereby having command on NH_3 and N_2O emissions (*Ding et al., 2015*). Denitrification losses depend on soil moisture, soil temperature, and N-ions availability. Even though NBPT has no direct effect on lowering denitrification losses, it was observed that it had influenced the reduction of emission of N_2O (*Cassim et al., 2021*). The reduction in the availability of NO_3^- has controlled the processes of denitrification and N_2O emission mainly in summer. In winter wheat, the primary controllers of denitrification processes are soil aeration and temperature (*Aulakh et al., 2001*).

Amending bio fertilizers like Azotobacter and VAM has positive effect on soil biomass carbon and soil biomass nitrogen. These microbes that help decomposition organic matter release the essential nutrients for plants to uptake there increasing the MBC and MBN. Amending biofertilizers like Azotobacter and VAM positively affect soil biomass carbon and nitrogen. Similar results were observed by Faujdar, (*2011*) while working on the effect of FYM, biofertilizers, and zinc on nutrients transformations, soil properties, and yield of maize, and their residual effect on wheat.

The soil urease enzyme was suppressed by N inhibitors by competitive inhibition mechanism there by reducing or slowing down the activity of the enzyme. Rapid losses in N are mainly due to urea hydrolysis by the urease enzyme, which drastically increases pH and NH_4^+ ion concentration in the soil (*Liu et al., 2018*). So this rise in pH and ion concentration increases NH_3 volatilization losses. In the entire growth period of wheat, the urease activity in the soil is always at a peak during tillering; as it grows to maturity, its activity is dropped. Similar results were reported by Fu et al. (*2019*) while working on the effects of urease and nitrification inhibitors on the soil.

The physiological characters of wheat analyzed (photosynthesis rate, chlorophyll content, LAI and stomatal conductance) also significantly influenced by organic and inorganic amendments. In this study, photosynthesis rate, chlorophyll content, LAI and stomatal conductance were found to be slightly increased in T_7 treatment with azotobacter because of increase in nitrogen availability which has a positive effect on these physiological parameters. Followed by Azotobacter treated plot, NBPT treated plot also showed significant improvement in all of these parameters.

The increase in the N content in wheat straw and grain might be due to improved nitrogen availability due to a reduced N loss and better NUE. As nitrogen is the most limiting nutrient in growth and development of the wheat crop. Better uptake of the nutrient N will affect the plant growth which will helps to attain the global food security by increasing the wheat yield by 1.5% per year. These results were found similar to the findings obtained by Singh et al. (*2018*) while working on certain microorganisms like *Bacillus* and *Azotobacter.* Mukhtar, Bashir & Nawaz (*2018*) found that microorganisms enhance crop growth by making nutrients available by fixing nitrogen and phosphate solubilization. *Azotobacter* can produce a few metabolites like phytohormones and exopolysaccharides, which help crops absorb nutrients and develop roots (*Hindersah et al., 2020*). Both are inorganic amendments that have effectively improved the nutrient availability to the plants, thereby increasing the yields. These findings were similar to those found by Kumar et al. (*2015*), who reported an increase in grain and biological yield by 22.6% and 17.4%, respectively. It was also reported by Galindo et al. (*2020*) that NBPT has some effect on metabolic pathways in decreasing urease enzyme activity hence increasing the NUE. Slowing down of nutrient release has improved the period of availability N and increased the crop's N uptake (*Liu et al., 2020*). These positive effects in turn enhanced the growth, development and yields of wheat. However, in reducing NH_3 volatilization NBPT, HQ amendments performed much better than other amendments but these amendments showed some negative effect on soil microorganism's activity.

Conclusions

The current study concludes that both organic and inorganic amendments significantly reduce NH_3 losses, thus increasing soil available nutrients and enhancing the NUE of the crops. Treatment containing NBPT reduced the losses by 40% as compared to only RDN without any amendments. These results are may be due to strong inhibition of urease activity by NBPT in the soil. Among plant-based amendments, garlic powder treated urea showed better results in decreasing NH_3 volatilization losses by 17% compared as to only RDN treated urea. These positive effects, in turn, enhanced the growth, development, and yields of wheat. However, in reducing NH_3 volatilization, inorganic amendments performed much better than organic amendments, but inorganic amendments showed some adverse effects on soil microorganism's activity. Thus, the focus should be more on the organic amendments rather inorganic amendments to the reduction of NH_3 emissions from agricultural fields.

Acknowledgements

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Table 1 (on next page)

graphical abstract

graphical abstract

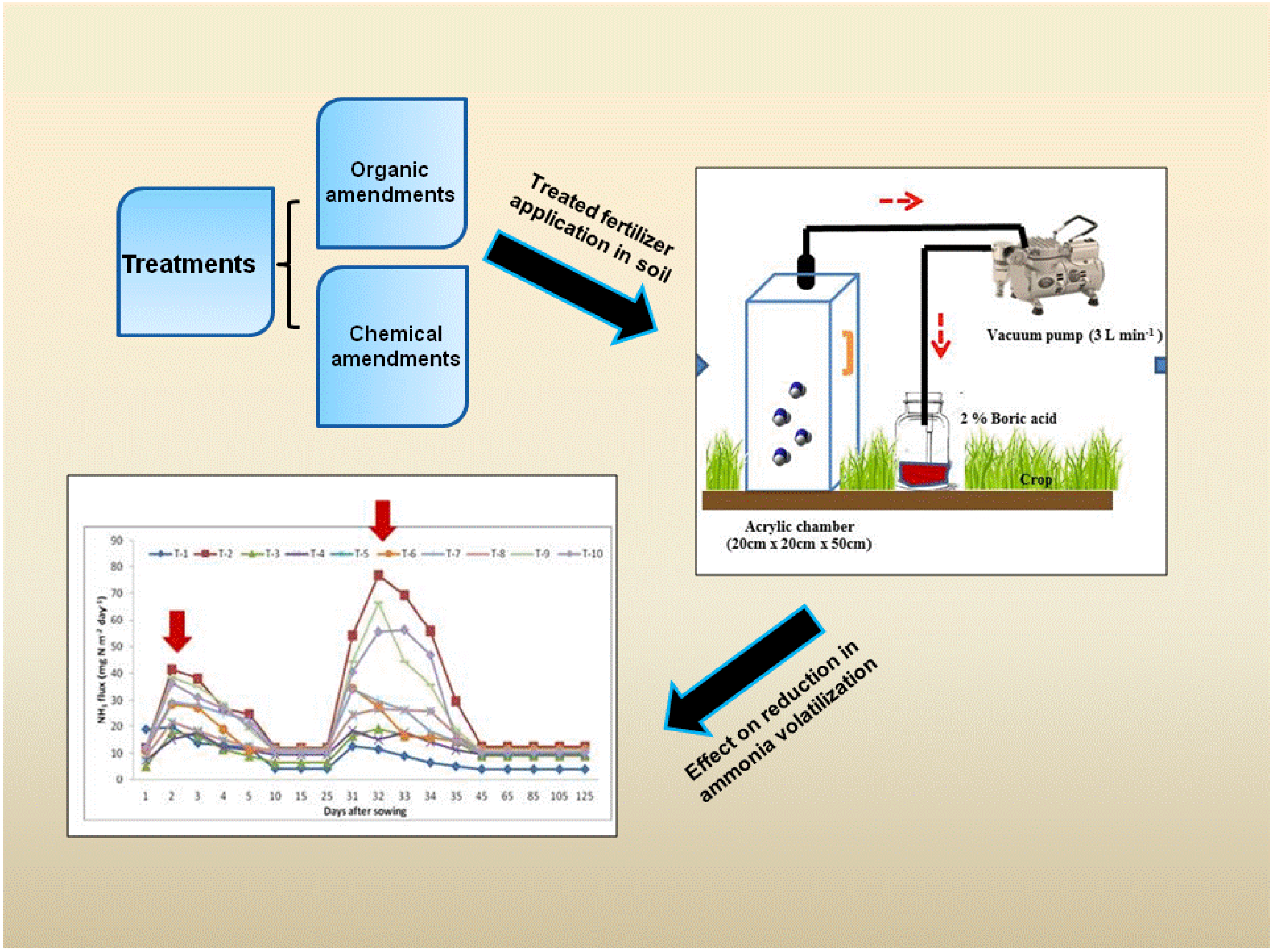
Treatments
Organic amendments
Chemical amendments
Treated fertilizer application in soil
Vacuum pump (3 L min-1)
2 % Boric acid
Crop
Acrylic chamber
(20cm x 20cm x 50cm)
Effect on reduction in ammonia volatilization
T-1 T-2 T-3 T-4 T-5 T-6 T-7 T-8 T-9 T-10
Days after sowing

Table 2(on next page)

Effect of different amendments on available nitrogen, phosphorous, potassium and soil organic carbon in soil, N concentration in wheat grains and straw

Effect of different amendments on available nitrogen, phosphorous, potassium and soil organic carbon in soil, N concentration in wheat grains and straw (statistically significant at p=0.05. Means followed by common alphabets are not significantly different among themselves by DMRT)

Treatments	Available N (kg ha^{-1}) after harvesting of the crop	Available P (kg ha^{-1}) after harvesting of the crop	Available K (kg ha^{-1}) after harvesting of the crop	Nitrogen content in grain (%)	Nitrogen content in straw (%)	Agronomic Efficiency of N (kg ha^{-1})	Production Efficiency of N (kg kg^{-1})	Apparent N Recovery (%)
T-1	141^{g}	17.9^{g}	214^{c}	1.5^{g}	0.32^{h}	0	0	0
T-2	173^{def}	19.7^{f}	221^{b}	1.91^{f}	0.39^{g}	9.1	25.8	35.2
T-3	172^{ef}	22.5^{e}	222^{b}	2.09^{c}	0.65^{a}	12.9	19.8	65.2
T-4	171^{f}	23.7^{d}	222^{b}	2.06^{cde}	0.62^{b}	11.6	18.5	62.8
T-5	177^{cde}	23.7^{de}	223^{b}	2.02^{de}	0.56^{c}	9.8	19.8	49.4
T-6	187^{b}	30.4^{a}	228^{a}	1.97^{b}	0.53^{d}	9.1	16.5	55.4
T-7	195^{a}	27.8^{b}	229^{a}	1.98^{a}	0.63^{ab}	12.5	15.4	80.8
T-8	180^{c}	26.9^{cb}	229^{a}	1.92^{b}	0.54^{d}	10.1	17.4	57.7
T-9	181^{cb}	27.58^{bc}	227^{a}	2.02^{cd}	0.41^{f}	9.6	21.5	44.8
T-10	178^{cd}	26.8^{c}	229^{a}	1.91^{e}	0.44^{e}	10.7	24	44.5
Statistical Significance (F test)	**Significant**	**Significant**	**Significant**	**Significant**	**Significant**	-	-	-
C.D.	5.55	1.58	3.30	0.10	0.02	-	-	-
SE(m)	1.85	0.53	1.10	0.03	0.01	-	-	-
SE(d)	2.62	0.75	1.56	0.05	0.01	-	-	-
C.V.	1.83	3.70	0.85	2.88	2.20	-	-	-

Table 1. Effect of different amendments on available nitrogen, phosphorous, potassium and soil organic carbon in soil, N concentration in wheat grains and straw (statistically significant at p=0.05. Means followed by common alphabets are not significantly different among themselves by DMRT)

Table 3 (on next page)

Effect of different amendments on yield attributes and yield of wheat. Mean of straw yield, biological yield, No. of tillers, spikes, grains per spike and harvest index

Effect of different amendments on yield attributes and yield of wheat. Mean of straw yield, biological yield, No. of tillers, spikes, grains per spike and harvest index (NS - Non-significant (P = 0.05) and mean of grain yield and test weight were found S-Significant (P=0.05) when compared to F-table value. Duncan's Multiple Range Test (DMRT) for comparison of mean yields and test weight of different amendment treatments (α=0.05) was conducted. Means of grain yield and test weight of different treatments followed by different alphabets are significantly different among themselves and Means with the same letter are not significantly different.)

Treatments	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Biological yield (t ha^{-1})	No. of tillers	No. of spikes	No. of grains per spike	Harvest Index	Test weight (g)
T-1	3.14^{c}	7.95	11.09	340.67	302.67	36.00	28.34	34.82 ^{b}
T-2	4.50^{ab}	8.85	13.35	448.67	423.67	40.00	33.72	39.35^{a}
T-3	5.09^{ab}	7.82	12.90	410.67	374.00	40.00	39.41	39.53^{a}
T-4	4.89^{a}	9.44	14.33	483.33	456.00	39.33	34.12	39.07^{a}
T-5	4.61^{ab}	8.16	12.77	432.33	402.33	40.67	36.09	39.40^{a}
T-6	4.51^{b}	8.30	12.81	403.67	370.67	40.67	35.21	39.58^{a}
T-7	5.01^{ab}	8.40	13.42	448.00	419.00	41.33	37.37	39.53^{a}
T-8	4.65^{ab}	8.42	13.07	448.00	416.00	38.67	35.59	39.76^{a}
T-9	4.59^{ab}	8.42	13.01	459.00	428.33	40.00	35.29	39.53^{a}
T-10	4.74^{ab}	8.23	12.97	466.00	430.00	40.00	36.57	39.16^{a}
Statistical Significance	**S**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**S**
C.D.	**0.76**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**1.34**
SE(m)	**0.25**	**0.99**	**1.05**	**3.25**	**33.98**	**33.40**	**1.03**	**0.54**
SE(d)	**0.36**	**1.40**	**1.49**	**4.59**	**48.05**	**47.24**	**1.45**	**0.77**
C.V.	**9.59**	**20.41**	**14.08**	**15.86**	**13.56**	**14.22**	**4.49**	**2.41**

Table 2. Effect of different amendments on yield attributes and yield of wheat. Mean of straw yield, biological yield, No. of tillers, spikes, grains per spike and harvest index

(NS - Non-significant (P = 0.05) and mean of grain yield and test weight were found S-Significant (P=0.05) when compared to F-table value. Duncan's Multiple Range Test (DMRT) for comparison of mean yields and test weight of different amendment treatments (α=0.05) was conducted. Means of grain yield and test weight of different treatments followed by different alphabets are significantly different among themselves and Means with the same letter are not significantly different.)

Figure 1

Figure 1. Temporal graph of ammonia flux and effect of organic and inorganic amendments on ammonia flux.

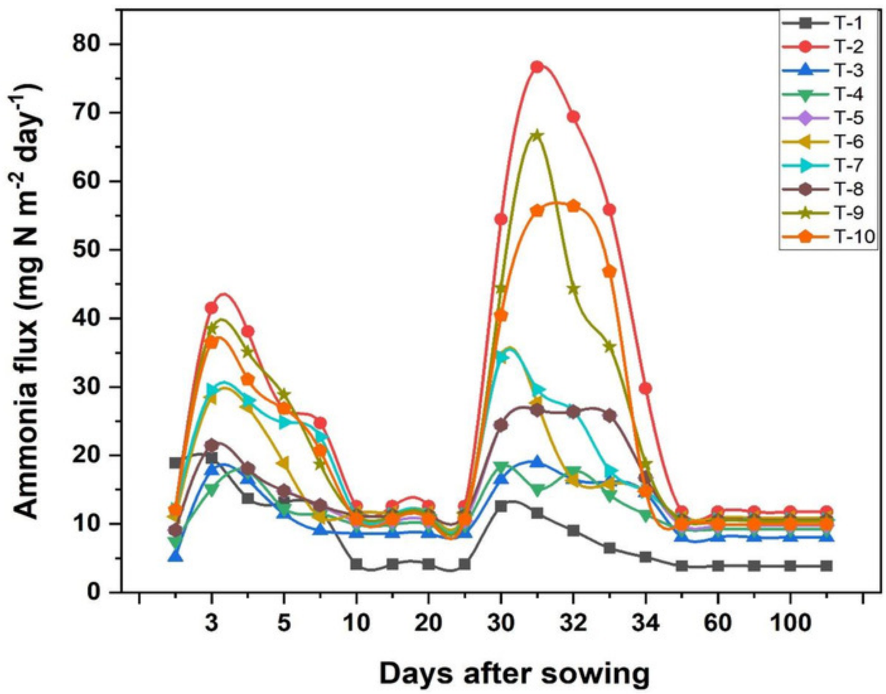

Figure 1. Temporal graph of ammonia flux and effect of organic and inorganic amendments on ammonia flux.

Figure 2

Figure 2. Effect of various organic and inorganic amendments on ammonia volatilization losses from the soil

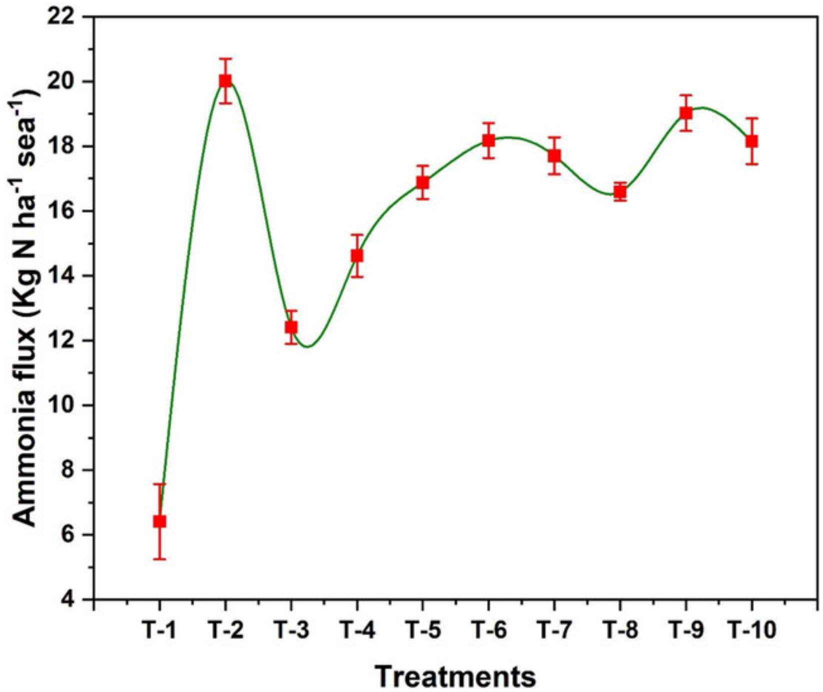

Figure 2. Effect of various organic and inorganic amendments on ammonia volatilization losses from the soil

Figure 3

Figure.3 Effect of various organic and inorganic amendments on soil organic carbon (SOC) in the soil after the harvesting of wheat

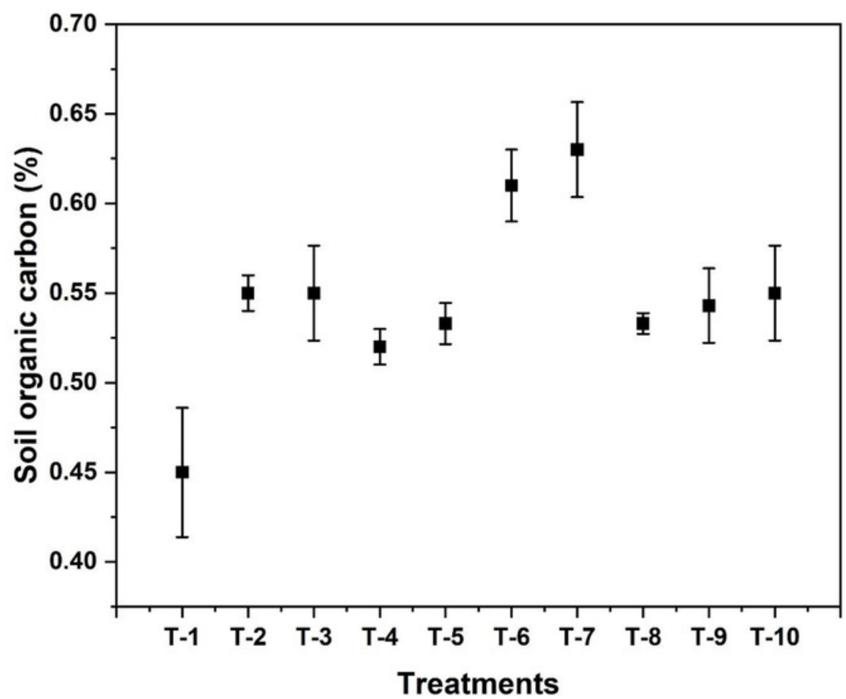

Figure.3 Effect of various organic and inorganic amendments on soil organic carbon (SOC) in the soil after the harvesting of wheat

Figure 4

Figure 4. Effect of various organic and inorganic amendments on NH_4^+-N and NO_3-N concentration in soil

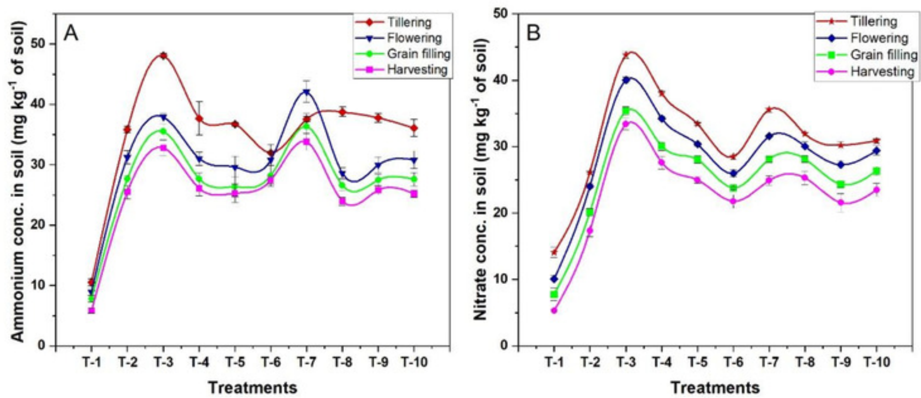

Figure 2.Effect of various organic and inorganic amendments on NH_4^+-N and NO_3-N concentration in soil

Figure 5

Figure 5. Effect of various organic and inorganic amendments on nitrous oxide flux and denitrification losses in soil

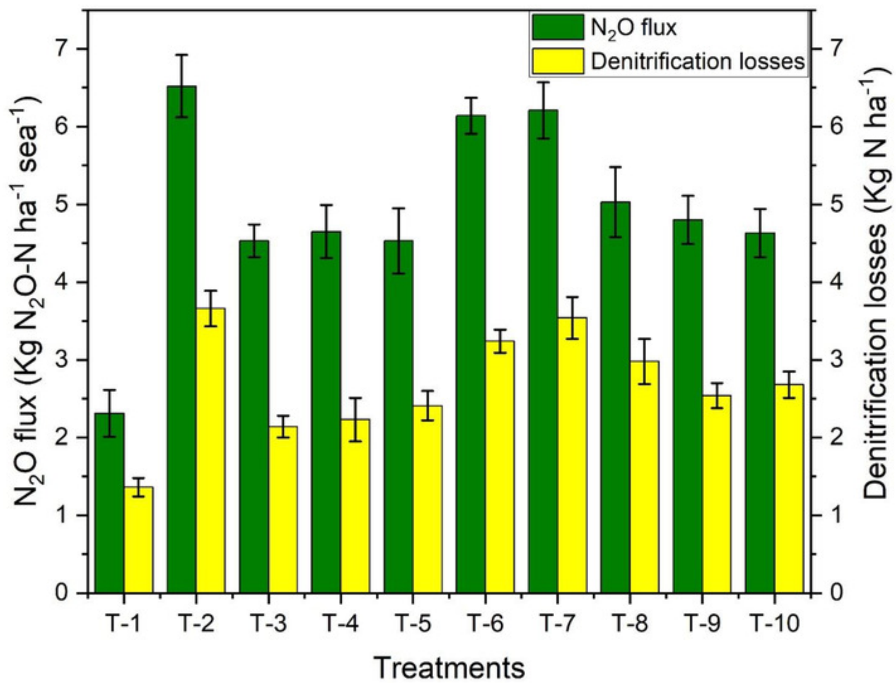

Figure 5. Effect of various organic and inorganic amendments on nitrous oxide flux and denitrification losses in soil

Figure 6

Figure 6. Effect of various organic and inorganic amendments on soil microbial biomass nitrogen and carbon (MBN and MBC) in soil

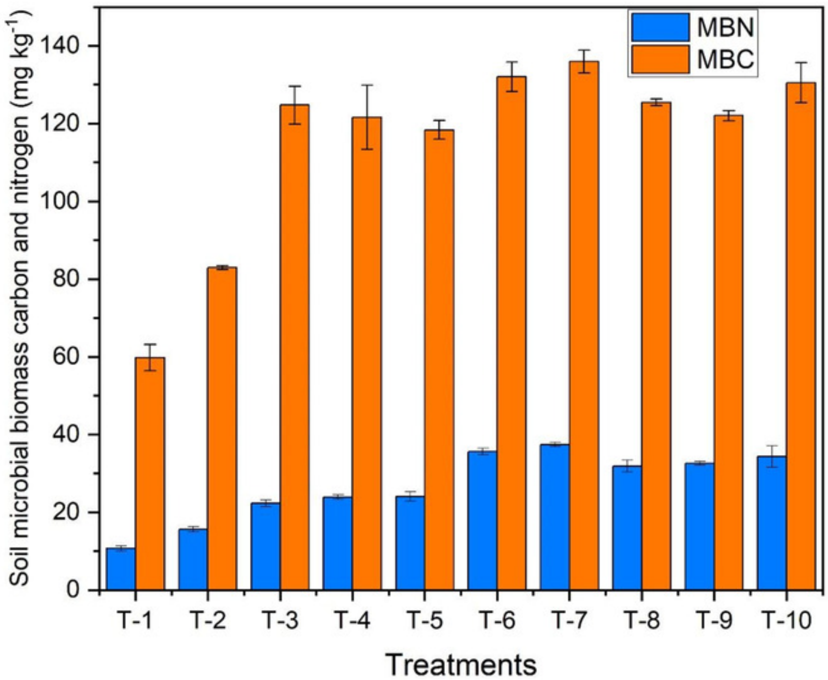

Figure 6. Effect of various organic and inorganic amendments on soil microbial biomass nitrogen and carbon (MBN and MBC) in soil

Figure 7

Figure 7. Effect of various organic and inorganic amendments on soil urease activity in wheat field

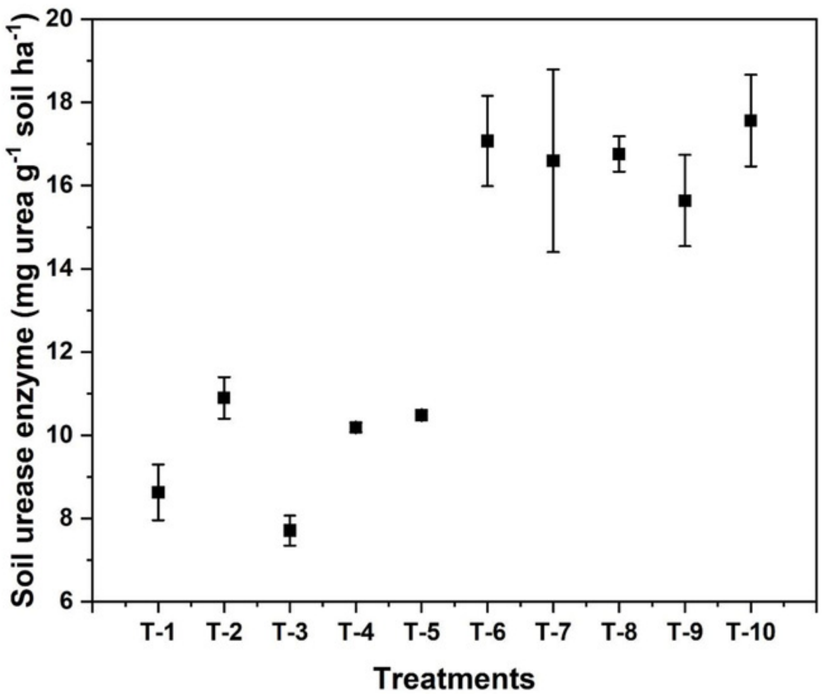

Figure 7. Effect of various organic and inorganic amendments on soil urease activity in wheat field