

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

Pooja L Ramalingappa¹, Manoj Shrivastava¹, Shiva Dhar², Kalikinkar Bandyopadhyay³, Shiv Prasad¹, Sapna Langyan⁴, Ritu Tomer¹, Ashish Khandelwal¹, Sibananda Darjee¹, Renu Singh^{Corresp. 1}

¹ Division of Environment Science, ICAR-Indian Agricultural Research Institute, Delhi, India

² Division of Agronomy, ICAR-Indian Agricultural Research Institute, Delhi, India

³ Division of Physics, ICAR-Indian Agricultural Research 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 (NH₃) 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.*, T₁-without nitrogen (control), T₂-Recommended Dose of Nitrogen (RDN), T₃-(N-(n-butyl) thiophosphoric triamide (NBPT @ 0.5% w/w of RDN), T₄-Hydroquinone (HQ @ 0.3% w/w of RDN), T₅-Calcium carbide (CaC₂ @ 1% w/w of RDN), T₆-Vesicular Arbuscular Mycorrhiza (VAM @ 10 kg ha⁻¹), T₇-(Azotobacter @ 50 g kg⁻¹ seeds), T₈-(Garlic powder @ 0.8% w/w of RDN), T₉-(Linseed oil @ 0.06% w/w of RDN), T₁₀-(Pongamia oil @ 0.06% w/w of RDN). **Results.** The highest NH₃ volatilization losses were observed in T₂ of about 20.4 kg ha⁻¹ per season. Significant reduction in NH₃ volatilization losses was observed in T₃ by 40%, T₄ by 27%, and T₈ by 17% when compared to control treatment. Soil urease activity was found to be decreased in plot receiving amendments, T₃, T₄, and T₅. The highest grain yield was observed in the T₇ treated plot with 5.09 t ha⁻¹ and straw yield of 9.44 t ha⁻¹ in T₄. **Conclusion.** The shifting towards inorganic and organic amendments is a feasible option to reduce NH₃ volatilization from wheat cultivation and improves NUE.

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

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6 Prasad¹, Sapna Langyan⁴, Ritu Tomer¹, Ashish Khandelwal¹, Sibananda Darjee¹, and Renu
7 Singh¹

8 ¹Division of Environment Science, ICAR-Indian Agricultural Research Institute, New Delhi,
9 110012, India

10 ²Division of Agronomy, ICAR-Indian Agricultural Research Institute, New Delhi 110012,
11 India

12 ³Division of Agricultural Physics, ICAR-Indian Agricultural Research Institute, New Delhi,
13 110012, India

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

16

17 Corresponding Author:

18 Renu Singh¹

19 Division of Environment Science, ICAR-Indian Agricultural Research Institute, New Delhi,
20 110012, India

21 Email address: renu_icar@yahoo.com

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31 Abstract

32 **Background.** This study focuses to investigate the effect of organic and inorganic
33 supplements on the reduction of ammonia (NH_3) volatilization, improvement in Nitrogen Use
34 Efficiency (NUE), and wheat yield.

35 **Methods.** A field experiment was conducted following Randomized Block Design with 10
36 treatments *i.e.*, T₁-without nitrogen (control), T₂-Recommended Dose of Nitrogen (RDN), T₃-
37 (N-(n-butyl) thiophosphoric triamide (NBPT @ 0.5% w/w of RDN), T₄-Hydroquinone (HQ
38 @ 0.3% w/w of RDN), T₅-Calcium carbide (CaC_2 @ 1% w/w of RDN), T₆-Vesicular
39 Arbuscular Mycorrhiza (VAM @ 10 kg ha⁻¹), T₇-(Azotobacter @ 50 g kg⁻¹ seeds), T₈-(Garlic
40 powder @ 0.8% w/w of RDN), T₉-(Linseed oil @ 0.06% w/w of RDN), T₁₀-(Pongamia oil @
41 0.06% w/w of RDN).

42 **Results.** The highest NH_3 volatilization losses were observed in T₂ of about 20.4 kg ha⁻¹ per
43 season. Significant reduction in NH_3 volatilization losses was observed in T₃ by 40%, T₄ by
44 27%, and T₈ by 17% when compared to control treatment. Soil urease activity was found to be
45 decreased in ^{plots} receiving amendments, T₃, T₄, and T₅. The highest grain yield was observed
46 in the T₇ treated plot with 5.09 t ha⁻¹, and straw yield of 9.44 t ha⁻¹ in T₄.

47 **Conclusion.** The shifting towards ^{why inorganic; wasn't organic better?} inorganic and organic amendments is a feasible option to
48 reduce NH_3 volatilization from wheat cultivation and improves NUE.

49

50 Introduction

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

61 volatilization risks range from 0.9 to 64 % of the applied N (a mean of 17.6 %) (*Pan et al.*,
62 2016). Ammonia volatilization into the atmosphere negatively affects agriculture, ecosystems,
63 and human health. Further, it also increases the loss of nitrogen for plant growth, thus
64 increasing the cost of cultivation (*Brink & Van Grinsven, 2011*). Secondary particulate matter
65 (PM10) is formed when NH_3 reacts with other air contaminants such as sulfuric acid and nitric
66 acid. It flies long distances and remains in the air for several days, and causes respiratory
67 diseases in humans (*Bittman et al., 2014*).

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

92 volatilization losses and enhance the productivity and NUE of the wheat crop. The application
93 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
94 losses and thus increasing the efficiency of crop nitrogen usage (*Abalos et al. 2014*). Different
95 mechanisms are involved in reduction of NH₃ volatilization in that NBPT retards the activity
96 of urease enzyme by competitive inhibition (*Fan et al., 2018*). Hydroquinone and Calcium
97 carbide are also effective in reducing the urease activity. Application of HQ to the soil not
98 only inhibited urease enzyme activity but also inhibited or enhanced the activity of other
99 enzymes like polyphenol oxidase, dehydrogenase, protease and phosphatase (*Wolejko et al.,*
100 *2020*). The CaC₂ has negative impact on ammonia oxidizing bacteria which are present in the
101 soil, also reduces the activity of dehydrogenase and nitrate reductase enzyme there by
102 reducing the N losses (*Mahmood et al., 2014*). Use of pongamia oil (karanjin) as a nitrogen loss
103 inhibitor works mainly by inhibiting the nitrification by reducing the activity of nitrosomonas
104 without affecting the activity of nitrobacter. The treatment with garlic extract which is rich in
105 compound thiosulfinate works on the mechanism of competitive inhibition of urease enzyme as it
106 contains a organosulphur group (-S(O)-S) which is similar to urea which helps in inhibiting
107 hydrolysis process (*Mathialagan et al., 2017*).

108 The world's population will surpass 9.7 billion by 2050, posing a significant obstacle to
109 achieving food sustainability. The projected increase in the world's population demands at
110 least a 70% increase in agriculture production in developed countries and 100% in developing
111 countries (*Mahmud et al., 2021*). Food security of the country and N-based global warming
112 and environmental degradation are interlinked (*Bilal & Aziz, 2022*). The use of urease
113 inhibitors in agricultural activities has already been investigated as one of the best methods for
114 ensuring adequate nutritional security (*Modolo et al., 2018*). N-(n-butyl) thiophosphoric
115 triamide treated urea lowers NH₃ loss by approximately 53%. The yield benefit from NBPT
116 application is 6.0 % and ranges from 0.8 to 10.2 % depending on crop types (*Cantarella et al.,*
117 *2018*). The effectiveness of NBPT in reducing NH₃ loss is well known, but there is still room
118 for progress to increase the amount of inhibition and hence the retention of NBPT-treated
119 urea in the field. However, these inorganic amendments are not economically viable, and most
120 of them restricted their usage in the research areas due to high cost. Upadhyay, Tewari &
121 Patra, (2011) investigated that these chemicals are harmful and inhibit the growth of specific
122

123 beneficial soil microorganisms that indirectly affects crop growth and development; thereby,
124 the following best option stands ^{for use of} are organic amendments.

125 Organic amendments are eco-friendly, economically viable, and, most importantly,
126 biodegradable. Therefore, they act as promising N inhibitors. There are many plant-derived
127 inhibitors like neem (*Azadirachta indica*) oil, pongamia (*Pongamia glabra*) oil, linseed
128 (*Linum usitatissimum*) oil, garlic extract, and mint (*Mentha spicata*) as N amendments in
129 inhibiting N losses. Thiosulfinates (TS) present in fresh garlic extract ^{acts as a} bio-inhibitor of
130 urease enzyme activity ^{and can be used as a} can be used as potential urease inhibitor in agriculture. Thiosulfinates
131 has potential to inhibit urease enzyme in 200 minutes after application (*Ramli et al., 2014*).

132 Application of coated urea fertilizers such as neem coated urea and pine oleoresin coated urea
133 in a vertisol reduced the NH₃ volatilization by 27.5% and 41.1%, respectively (*Jadon et al.,*
134 *2018*). But these organic amendments lack commercialization and efficient utilization. So this
135 study concentrates mainly on stressing the importance of these organic amendments in
136 comparison with inorganic amendments and decreases the contamination of soil and
137 environment in parallel with increasing NUE.

138 With this interest, a study on “Reducing ammonia volatilization using organic and inorganic
139 amendments in Wheat (*Triticum aestivum* L.)” was studied with two objectives. ^{The first} First
140 objective ^{was to} is to study the effect of organic and inorganic amendments on ammonia
141 volatilization ^{and the} and second one ^{was to} is to evaluate the effect of organic and inorganic amendments on
142 the NUE and productivity of wheat. We hypothesize that the organic and inorganic
143 amendments have the potential to reduce NH₃ volatilization, increase the NUE and yield of
144 wheat.

145

146 **Materials & Methods**

147 **Details of the experimental site**

148 A field study was taken at the research farm of the Indian Agricultural Research Institute,
149 New Delhi, located at 28° 40 'N and 77° 12' E, at an altitude of 228.16 m above mean sea
150 level (sub-tropical and semi-arid region), during November 2020 to April 2021. The
151 Southwest monsoon contributed about 80% of this rainfall, with an average of 650 mm
152 annually. The pH of the soil was 8 (±0.1), with a sandy clay loam texture. The initial soil

153 available N was low (157 ± 0.5 kg ha⁻¹), medium level of available P (13.5 ± 0.2 kg ha⁻¹) and
154 available K (196 ± 0.8 kg ha⁻¹).

155 **Experimental design and management**

156 The experiment was conducted in a randomized block design (RBD) with 3 replications, and
157 each plot area was 12 m² (4 m×3 m). In all treatments recommended dose of nitrogen (RDN)
158 was applied except T₁ (control), where no RDN was used. The research was carried out with
159 growing wheat variety (HD 2967) with 10 treatments in respective plots namely labeled as T₁ (control), T₂
160 (RDN @ 150 kg ha⁻¹), T₃ (RDN + NBPT (N-(n-butyl) thiophosphoric triamide) @ 0.5% w/w
161 RDN), T₄ (RDN + HQ (Hydroquinone) @ 0.3% w/w of RDN), T₅ (RDN + Calcium Carbide
162 @ 1% w/w of RDN), T₆ (RDN + VAM (Vesicular Arbuscular Mycorrhiza @ 10 kg ha⁻¹), T₇
163 (RDN + Azotobacter @ 50 g kg⁻¹ seeds) T₈ (RDN + Garlic powder @ 0.8% w/w of RDN), T₉
164 (RDN + Linseed oil @ 0.06% w/w of RDN), and T₁₀ (RDN + Pongamia oil @ 0.06% w/w of
165 RDN). Each dose was finalized based on the current usage of these amendments by firms,
166 which were applied in 2 splits (One at the time of sowing and the second split application 30
167 days after sowing). To study the effect of organic and inorganic amendments together three
168 inorganic amendments, two biofertilizers and three organic amendments were chosen based on
169 popularity and review of literatures. N-(n-butyl) thiophosphoric triamide (*Zanin et al., 2015*),
170 Hydroquinone (*Modolo et al., 2018*), Calcium Carbide (*Sakariyawo et al., 2020*), Garlic
171 powder (*Ramli et al., 2014*), Linseed oil, and Pongamia oil (*Majumdar, 2008*) were mixed
172 with Urea (RDN) as per dosage. Wheat seeds were treated with *Azotobacter spp.* (as per ICAR
173 RABI Agro-Advisory For Farmers) and used for sowing in T₇. Vesicular Arbuscular
174 Mycorrhiza (as per ICAR RABI Agro-Advisory For Farmers) was applied directly into the
175 soil during sowing with a RDN. The recommended dose of fertilizers (RDF) for wheat was
176 150:60:40 kg ha⁻¹ N: P₂O₅:K₂O. Urea, single super phosphate (SSP), and muriate of potash
177 (MOP) were used as a source of N, P₂O₅, and K₂O, respectively.

178 **Method of Soil sample collection and analysis**

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

184 **Collection and analysis of NH₃**

185 The ammonia volatilization was monitored after fertilizer application up to 10 days by using
186 a forced-air Forced air draft system method (*Bhaskar et al. 2022; Stumpe et al. 1984; Bremner, 1965*).

187 The closed-chambers measuring 20 cm × 20 cm × 50 cm size made of 6 mm acrylic sheets
188 were placed in the field. The volatilized NH₃ gas from soil surface under different treatments
189 were collected in a 2 % Boric acid solution containing mixed indicator (methyl red and
190 bromocresol green). The air inside the chamber was collected into boric acid traps using a
191 vacuum pump having a flow rate of 3 L min⁻¹. The boric acid traps were changed every
192 24 hr. The volatilized NH₃ was determined by the titration of boric acid solution with 0.02
193 N sulphuric acid, and further the listed formula. further calculated using formula.

194 **Total Nitrogen and NH₄⁺-N and NO₃⁻-N analysis**

195 Total nitrogen content in soil was determined by the Kjeldahl method (*Kjeldahl, 1883*) during
196 the initial and after harvesting of crop growth. The NH₄⁺-N and NO₃⁻-N were analyzed
197 through a steam distillation method (*Keeney and Bremner, 1965*) during all four crop growth
198 stages (Tillering, Flowering, Grain-filling, Physiological maturity). The extract was prepared
199 by taking 10 g soils with 0.25 g activated charcoal and 50 mL KCl Solution and kept for
200 shaking (30 min) then filtered with Whatman filter paper 1. From the same extract 10 mL of
201 KCl extract was taken in two different distillation flask and 100 mL distilled water was added
202 to each flask. 1g Devardas alloy was added for the case of NH₄⁺-N estimation and for NO₃⁻-N
203 estimation 1.0 g magnesium oxide (MgO) was added and distilled separately and captured in
204 20 mL 2% Boric acid and titrated against 0.02 N sulfuric acid. Further calculation was done.
205 Available Phosphorus was analyzed using the Olsen method of estimation (*Olsen et al., 1954*).
206 Available P from soil sample was extracted using 0.5 N NaHCO₃ solution buffer at pH 8.5
207 then P in the extract was measured by ascorbic acid method using a spectrophotometer.
208 Available potassium in the soil were measured using an ammonium acetate method (*Hanway &*
209 *Heidel, 1952*), where available K was extracted by shaking with neutral normal ammonium
210 acetate for 5 min the K is determined using flame photometer. Soil Organic Carbon was
211 measured using Walkley and Black's rapid titration method (*Walkley & Black, 1934*).

212 **Analysis of Nitrous oxide (N₂O) and others significant parameters**

213 Nitrous oxide flux was analyzed using the closed chamber method (*Herr et al., 2020*). In this
214 method, dark PVC boxes were installed, and samples were drawn every 24 hrs in the morning

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215 using syringes and evacuated into plastic vials, and analyzed chromatographically.
 216 Denitrification losses were estimated by denitrification enzyme assay as described by Smith &
 217 Tiedje (1979).
 218 Soil urease activity was also analyzed at the flowering stage, calorimetrically by Bremner &
 219 Douglas (1971) method. Normalized Difference Vegetation Index (NDVI) was measured
 220 using Green seeker (handheld crop sensor by Trimble) at 50% flowering stage. IRGA (LI-
 221 COR Model LI-6400X7 portable photosynthetic system) was used to measure the
 222 photosynthetic rate and stomatal conductance. Soil Microbial Biomass Carbon (MBC) and
 223 Soil Microbial Biomass Nitrogen (MBN) were determined by the chloroform fumigation–
 224 extraction method described by Vance et al. (1987) and Brookes et al. (1985), respectively.
 225 The N content in grains and straw was also measured using the Kjeldahl method (Kjeldahl,
 226 1883). After harvesting of the crop, yield attributes were calculated from each plot.

227 **Calculation of Nitrogen Use Efficiency in wheat**

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

$$230 \text{ Agronomic Efficiency of N (AEN) (kg ha}^{-1}\text{)} = \frac{GYF - GYN}{AFN} \dots\dots\dots \text{Eq. 1}$$

$$231 \text{ Production Efficiency of N (PEN) (kg kg}^{-1}\text{)} = \frac{GYF - GYN}{TUN - CUN} \dots\dots\dots \text{Eq. 2}$$

$$232 \text{ Apparent N Recovery (ANR) (\%)} = \frac{TUN - CUN}{AFN} \times 100 \dots\dots\dots \text{Eq. 3}$$

233 TUN = Total N uptake from the fertilized plot (kg ha⁻¹)

234 CUN = Total N uptake from unfertilized / control plot (kg ha⁻¹)

235 AFN = Amount of applied fertilizer N (kg ha⁻¹)

236 GYF = Grain yield in the fertilized plot (kg ha⁻¹)

237 GYN = Grain yield in unfertilized/control plot (kg ha⁻¹)

238 **2.7 Statistical analysis**

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

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

246

247 **Results**

248 **Ammonia volatilization losses during the wheat growth period**

249 Additions of both organic and inorganic amendments have effectively controlled the ammonia
250 volatilization losses. Ammonia losses were measured during the initial 5 days after applying
251 fertilizer (*Bhaskar et al., 2022*), which was applied in 2 splits (0 DAS and 30 DAS). The
252 temporal graph shown in figure 1 depicts two peaks of NH₃ flux in the entire cropping season.
253 Significant losses through NH₃ volatilization were observed in the initial 5-6 days after
254 fertilizer application. The highest volatilization losses were observed in T₂ of about 20 kg ha⁻¹
255 season⁻¹ and the lowest was found to be in T₁ i.e. 6.4 kg ha⁻¹ season⁻¹. T₃ performed well in
256 reducing the losses by 40% when compared to the NH₃ losses in T₂. T₃, T₄ and T₈ showed the
257 lowest emissions of NH₃ among all the treatments by losing 12.4, 14.6, 16.6 kg ha⁻¹ season⁻¹,
258 respectively to the atmosphere (Figure 2). Among plant-based amendments, T₈ with garlic
259 powder treated urea showed better results by reducing NH₃ volatilization losses by 34%
260 compared to only RDF treated urea (T₂). Duncan Multiple Range Test revealed that T₃
261 (NBPT) and T₄ (HQ) were effective in reducing the NH₃ losses. T₅ (CaC₂) and T₈ (Garlic
262 powder) showed no significant difference among their means i.e had similar effect in reducing
263 NH₃ losses next to NBPT. Other treatments were not that effective in reducing the NH₃ losses.

264 **Initial and final soil nutrient status**

265 The total nitrogen content and available nitrogen content in the soil, when analyzed after
266 harvesting of the crop, were found to be highest in plot T₇, i.e., Azotobacter treated plot
267 (0.041% and 195 kg ha⁻¹, respectively), followed by T₆, i.e., VAM treated plot (0.039% and
268 187 kg ha⁻¹, respectively) Available N content for different treatments are given in table 1.
269 The highest SOC was observed in T₇, treated with azotobacter, i.e., 0.63%. It has significantly
270 increased the SOC content when compared to the initial SOC (0.42%) before sowing of the
271 wheat crop as shown in figure 3. The highest levels of P were observed in T₆ treated with
272 VAM, i.e., 30.4 kg ha⁻¹, which is more than double the amount of initial value of available P

273 as shown in table 1. The initial level of available K was 196 kg ha⁻¹, while in the final
274 analysis, it was increased up to 229 kg ha⁻¹ in T₁₀, as depicted in table 1.

275 **Ammonical N (NH₄⁺-N) and Nitrate (NO₃⁻-N) in soil**

276 The available nitrogen forms, majorly NH₄⁺-N and NO₃⁻-N in soil, are found to be decreasing
277 from tillering stage to the grain filling stage (Figure 4.). The mean quantity of NH₄⁺-N in soil
278 at various growth stages (tillering, flowering, grain filling stage, physiological maturity stage)
279 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
280 of NO₃⁻-N in soil at all four growth stages were found to be 70 kg ha⁻¹, 63.4 kg ha⁻¹, 56.5 kg
281 ha⁻¹, 51.1 kg ha⁻¹ respectively. During the tillering stage, the highest NH₄⁺-N (108 kg ha⁻¹)
282 was observed in T₃, i.e., treatment with NBPT amended urea, and the highest NO₃⁻-N (58.5 kg
283 ha⁻¹) was found in RDN treated plot (T₂). In all the stages of plant growth T₃ showed
284 significantly higher level of NH₄⁺-N which was 47% higher than T₂. Duncan Multiple Range
285 Test also showed that the mean of treatments T₄, T₇ and T₈ also significantly at par for
286 available NH₄⁺-N content in soil and in case of NO₃⁻-N availability, mean of treatments T₄,
287 T₇ and T₅ also showed significant difference during all the stages of the wheat crop growth.

288 **Nitrous oxide flux and denitrification losses from soil**

289 Nitrous oxide emission is directly related to the amount of N fertilizer available in the soil.
290 The highest cumulative N₂O flux was observed in T₂ (RDF) plots with 6.52 kg N₂O-N ha⁻¹
291 season⁻¹. There were no significant differences between T₃, T₄, and T₅ in the case of N₂O
292 emission reduction (Figure 3). But all of them were significant when compared with T₂.
293 Among plant-based inhibitor treatments, T₈, having urea amended with garlic powder, showed
294 effective results. The highest denitrification losses were observed in T₂ (3.66 kg-N ha⁻¹)
295 (Figure 5). N-(n-butyl) thiophosphoric triamide treated urea was comparatively effective by
296 reducing the denitrification losses by 35 %.

297 **Soil microbial biomass carbon and nitrogen in soil**

298 Soil biomass carbon, and soil biomass nitrogen (found within living organisms like fungi and
299 bacteria) were observed at the flowering stage of the crop. The highest MBN content was
300 reported in T₇, i.e., Azotobacter treated plot with 37 mg kg⁻¹, followed by T₆, having VAM
301 treated plot. Highest MBC (136 mg kg⁻¹) was observed in T₇, which is Azotobacter treated
302 plot, and it was 63.9% more than that of T₂ (Figure 6).

303 **Soil urease enzyme activity in soil**

304 As observed in figure 7, under the flowering stage in T_2 , i.e., only RDN treated plot showed
305 the highest urease activity ($18.9 \text{ mg urea g}^{-1} \text{ soil h}^{-1}$), and the lowest urease activity (7.71 mg
306 $\text{urea g}^{-1} \text{ soil h}^{-1}$) was observed in T_3 treatment containing NBPT which was indicated by the
307 effectiveness of urease inhibitor.

308 **Physiological parameters of wheat crop**

309 Addition of organic and inorganic amendments to the soil has significantly influenced the
310 physiological parameters (photosynthesis rate. The range of photosynthesis rate was
311 varied from $15.7 \mu \text{ mol m}^{-2} \text{ s}^{-1}$ to $23.8 \mu \text{ mol m}^{-2} \text{ s}^{-1}$ which was recorded during 50% flowering
312 stage. The highest photosynthetic rate was observed in T_7 with $23.8 \mu \text{ mol m}^{-2} \text{ s}^{-1}$ followed by T_3 ,
313 T_6 ($23.3 \mu \text{ mol m}^{-2} \text{ s}^{-1}$ and $22.9 \mu \text{ mol m}^{-2} \text{ s}^{-1}$). The range of chlorophyll content was varied
314 from 0.66 to 0.77 which was recorded during 50% flowering stage. The highest chlorophyll
315 content was observed in both T_4 and T_7 with 0.77 followed by T_6 and T_{10} showing 0.76. The
316 highest LAI was observed in T_7 with 4.59 followed by T_3 and T_8 which has recorded the same
317 LAI i.e. 4.16. The least reading was recorded in treatment T_1 i.e. 1.85. The highest stomatal
318 conductance was observed in T_7 with $0.48 \text{ m mol m}^{-2} \text{ s}^{-1}$ followed by T_3 , T_8 ($0.47 \text{ m mol m}^{-2} \text{ s}^{-1}$
319 1 and $0.43 \text{ m mol m}^{-2} \text{ s}^{-1}$), respectively.

320

321 **Nitrogen uptake by wheat**

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

327 **Wheat yield and nitrogen use efficiency**

328 The data obtained in grain and straw yield showed significant improvement in yields due to
329 the addition of organic and inorganic amendments. The highest grain yield was observed in
330 T_3 , i.e., treatment with NBPT amended urea with 5.09 t ha^{-1} , and straw yield was in T_4 , i.e.,
331 treatment with hydroquinone amended urea (Table 2) but in case of straw yield all the
332 treatment means were not significantly different at $p=0.05$. The other yield attributes like
333 biological yield, number of tillers per m^{-2} , number of spikes per m^{-2} , number of grains per
334 spike, and harvest index also were not significantly influenced by amendments addition

335 (p=0.05). The test weight of the wheat grain of treatment T₈ (39.8 g) was significant among all
336 the treatments. .

337 As nitrogen content in the grains has increased on amending urea with organic and inorganic
338 amendments, NUE was calculated in terms of AEN, PEN, and ANR using Equations 2, 3, and
339 4. The highest AEN was found in T₃ with 12.9 kg ha⁻¹, and the lowest was in T₂, 9.05 kg ha⁻¹
340 (Table 1). The highest PEN was observed in T₂ with 25.8 kg kg⁻¹, and the lowest was in T₇,
341 (15.4 kg kg⁻¹). The average value of PEN was 17.9 kg kg⁻¹. The highest ARN (81 %) was seen
342 in T₇, and the lowest was in T₂ (35 %). The mean value of ANR was observed to be 49.6%.

343

344 Discussion

345 Our major findings in case of NH₃ volatilization losses depicted that T₃ out performed
346 compared to all other treatments because of competitive inhibition of urease enzyme activity
347 by NBPT in the soil which has a major role in urea hydrolysis process where urea molecule is
348 converted into ammonium ion. Next to NBPT, the other inorganic amendments significantly
349 decreased the NH₃ volatilization losses. The allicin (thiosulfinate) naturally present in garlic is
350 a bio- inhibitor of urease activity, as it contains an organosulfur functional group similar to
351 that of the ureas. This might help allicin to decrease urease activity. This is in agreement with
352 the research study conducted by Mathialagan et al. (2017). These amendments have practical
353 implications in reducing the quantity of urea applied to the field there by reducing the N
354 losses. Even though many studies are taken up in case of NBPT, still there is need to improve
355 the duration of inhibition of urease activity, shelf life of urea fertilizer coated with NBPT and
356 economic viability of these compounds. Agriculture alone contributes 80-90% NH₃ emissions
357 globally mainly through volatilization from livestock and synthetic nitrogen fertilizer (*Xu et*
358 *al.*, 2019). It acts as pollutant influencing biosphere by formation of haze and soil
359 acidification. N₂O which is potential greenhouse gas also of a great concern which contributes
360 in global warming and affects human and environmental health. Li et al. (2015) reported that
361 the Limus[®] (a new urease inhibitor consisting of 75% NBPT and 25% N-(n-propyl)
362 thiophosphoric triamide (NPPT)), showed on an average 83% decrease in NH₃ losses during
363 winter wheat season in China. Only the addition of NBPT can reduce NH₃ volatilization losses
364 by 61% to 74%, as reported by Lasisi, Akinremi & Kumaragamage (2019). In his study on the
365 efficiency of winter versus spring-applied urea-based fertilizers treated with urease and

366 nitrification inhibitors. Affendi, Mansor & Samiri (2019) conducted a study by adding various
367 chemical and natural urease inhibitors to reduce ammonia and nitrous oxide losses from soil.
368 They reported similar findings in a combination of thiosulfate with urea. However, they found
369 NBPT was more effective than the combined use of thiosulfate with urea to reduce NH_3
370 volatilization losses. Similar results were reported by Eduardo et al. (2016), he reported a
371 decrease of 31.6% N, which used to be lost if not applied with thiosulfates, and concluded it
372 as an upcoming natural urease inhibitor.

373 The final analysis of TNC, available N, P and K reported increase in all these nutrient contents
374 than the initial analysis which helps the next sown crop in nutrition. T_7 treated with azotobacter
375 performed well in increasing N and K content in soil and T_6 treated with VAM in increasing P
376 content in soil. Increase in N content of the soil could be due to the addition of these bio
377 fertilizers as amendments that significantly increased residual N and reduced the N fertilizer
378 application for the next crop, which will be sown in that plots. Vesicular Arbuscular
379 Mycorrhiza improves the mobilization of P; hence uptake by wheat crop and P content in soil is
380 increased. Addition of biofertilizers as a source of amendments in order to reduce the N losses
381 has improved the SOC of the soil. The highest SOC was observed in T_7 which was treated with
382 azotobacter i.e. 0.63%. It has doubled the SOC content when compared to initial SOC before
383 sowing of wheat crop i.e. 0.42%. A similar effect of these bio fertilizers in increasing total
384 nitrogen content was observed in a study on Bio-fertilizer affects structural dynamics, function,
385 and network patterns of the sugarcane rhizospheric microbiota by Liu et al. (2021). Kader et al.
386 (2002) also found similar results while working on the effect of azotobacter inoculants on
387 wheat's yield and nitrogen uptake. This result is supported by Suri et al. (2011) while working
388 on the influence of VAM and applied P on root colonization in wheat.

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

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

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

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

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

425 The physiological characters of wheat analyzed (photosynthesis rate, chlorophyll content,
426 LAI and stomatal conductance) also significantly influenced by organic and inorganic
427 amendments. In this study, photosynthesis rate, chlorophyll content, LAI and stomatal

428 conductance were found to be slightly increased in T₇ treatment with azotobacter because of
429 increase in nitrogen availability which has a positive effect on these physiological
430 parameters. Followed by Azotobacter treated plot, NBPT treated plot also showed significant
431 improvement in all of these parameters.

432 The increase in the N content in wheat straw and grain might be due to improved nitrogen
433 availability due to a reduced N loss and better NUE. As nitrogen is the most limiting nutrient
434 in growth and development of the wheat crop. Better uptake of the nutrient N will affect the
435 plant growth which will helps to attain the global food security by increasing the wheat yield
436 by 1.5% per year. These results were found similar to the findings obtained by Singh et al.
437 (2018) while working on certain microorganisms like *Bacillus* and *Azotobacter*. Mukhtar,
438 Bashir & Nawaz (2018) found that microorganisms enhance crop growth by making nutrients
439 available by fixing nitrogen and phosphate solubilization. *Azotobacter* can produce a few
440 metabolites like phytohormones and exopolysaccharides, which help crops absorb nutrients
441 and develop roots (Hindersah et al., 2020). Both are inorganic amendments that have
442 effectively improved the nutrient availability to the plants, thereby increasing the yields. These
443 findings were similar to those found by Kumar et al. (2015), who reported an increase in grain
444 and biological yield by 22.6% and 17.4%, respectively. It was also reported by Galindo et al.
445 (2020) that NBPT has some effect on metabolic pathways in decreasing urease enzyme activity
446 hence increasing the NUE. Slowing down of nutrient release has improved the period of
447 availability N and increased the crop's N uptake (Liu et al., 2020). These positive effects in
448 turn enhanced the growth, development and yields of wheat. However, in reducing NH₃
449 volatilization NBPT, HQ amendments performed much better than other amendments but
450 these amendments showed some negative effect on soil microorganism's activity.

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456 **Conclusions**

457 The current study concludes that both organic and inorganic amendments significantly reduce
458 NH₃ losses, thus increasing soil available nutrients and enhancing the NUE of the crops.
459 Treatment containing NBPT reduced the losses by 40% as compared to only RDN without
460 any amendments. These results are may be due to strong inhibition of urease activity by
461 NBPT in the soil. Among plant-based amendments, garlic powder treated urea showed better
462 results in decreasing NH₃ volatilization losses by 17% compared as to only RDN treated urea.
463 These positive effects, in turn, enhanced the growth, development, and yields of wheat.
464 However, in reducing NH₃ volatilization, inorganic amendments performed much better than
465 organic amendments, but inorganic amendments showed some adverse effects on soil
466 microorganism's activity. Thus, the focus should be more on the organic amendments rather
467 inorganic amendments to the reduction of NH₃ emissions from agricultural fields.
468

469 **Acknowledgements**

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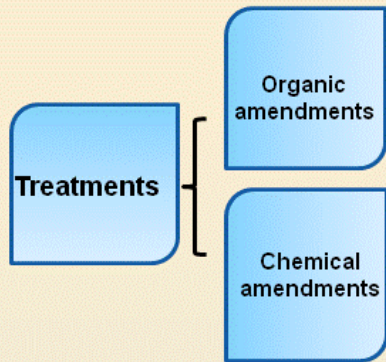
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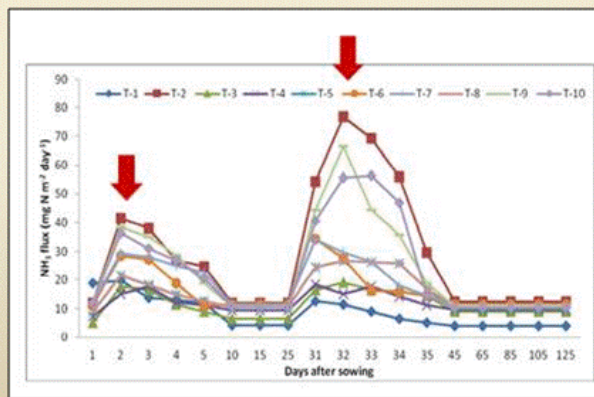
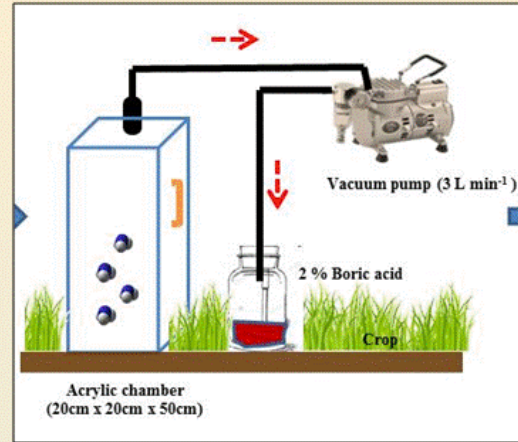
Table 1 (on next page)

graphical abstract

graphical abstract



Treated fertilizer application in soil



Effect on reduction in ammonia volatilization

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	-	-	-

1

2 **Table 1. Effect of different amendments on available nitrogen, phosphorous, potassium and soil organic carbon in soil, N**
3 **concentration in wheat grains and straw (statistically significant at p=0.05. Means followed by common alphabets are not**
4 **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 ($\alpha=0.05$) 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

1

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

4 (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
5 value. Duncan's Multiple Range Test (DMRT) for comparison of mean yields and test weight of different amendment treatments
6 ($\alpha=0.05$) was conducted. Means of grain yield and test weight of different treatments followed by different alphabets are significantly
7 different among themselves and Means with the same letter are not significantly different.)

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Figure 1

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

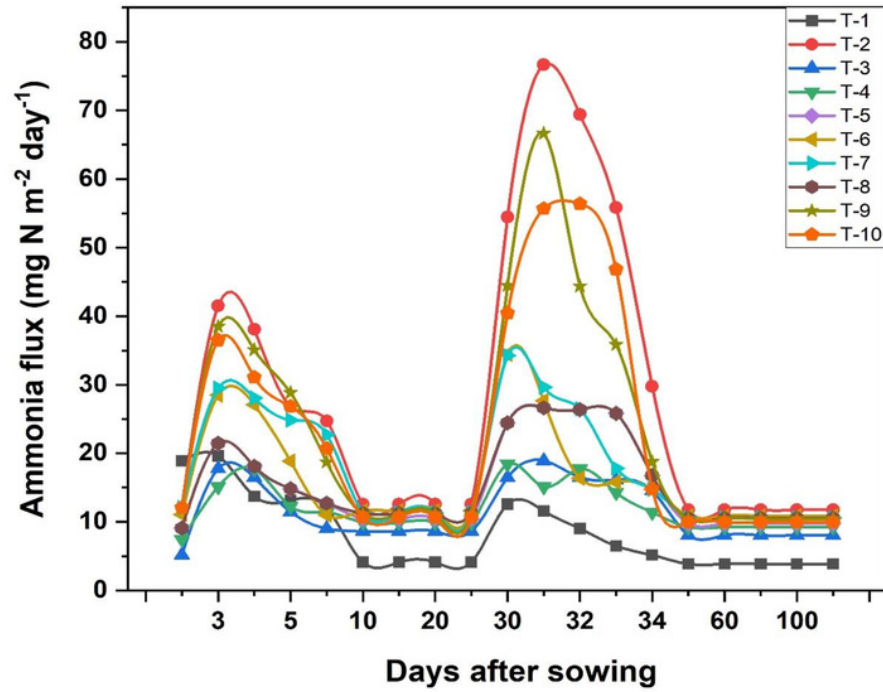


Figure 1. Temporal graph of ammonia flux and effect of organic and inorganic amendments on ammonia flux.

Figure 2

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

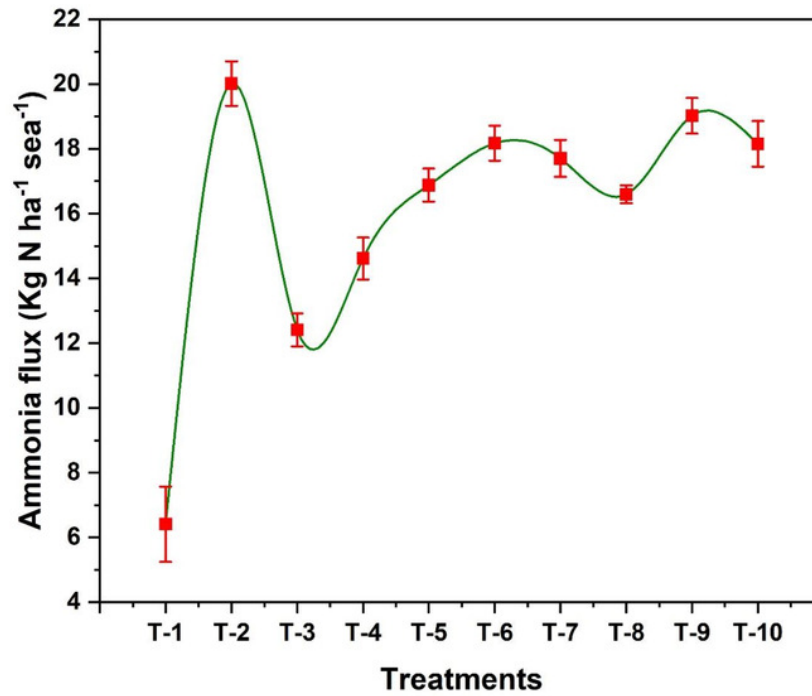


Figure 2. Effect of various organic and inorganic amendments on ammonia volatilization losses from the soil

Figure 3

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

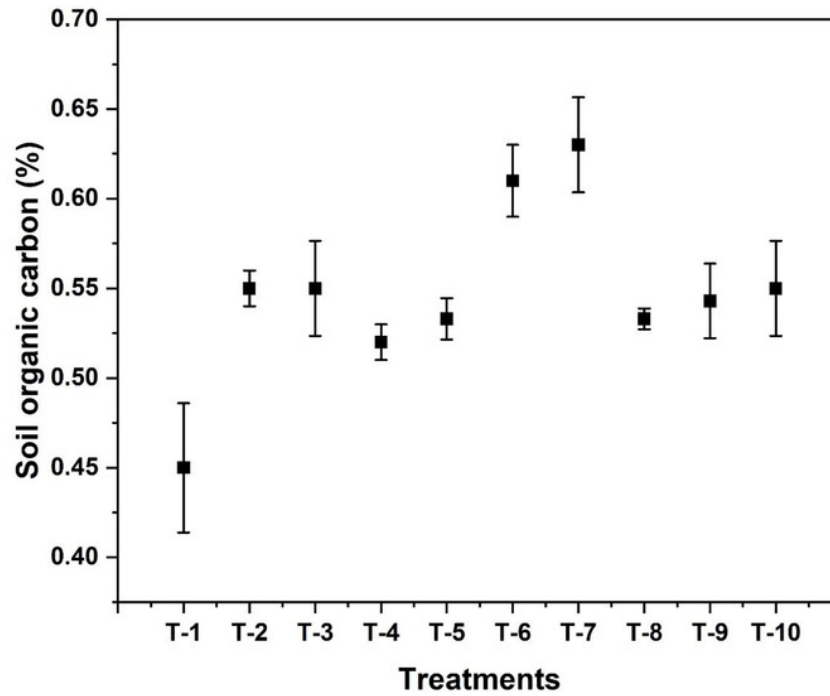


Figure.3 Effect of various organic and inorganic amendments on soil organic carbon (SOC) in the soil after the harvesting of wheat

Figure 4

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

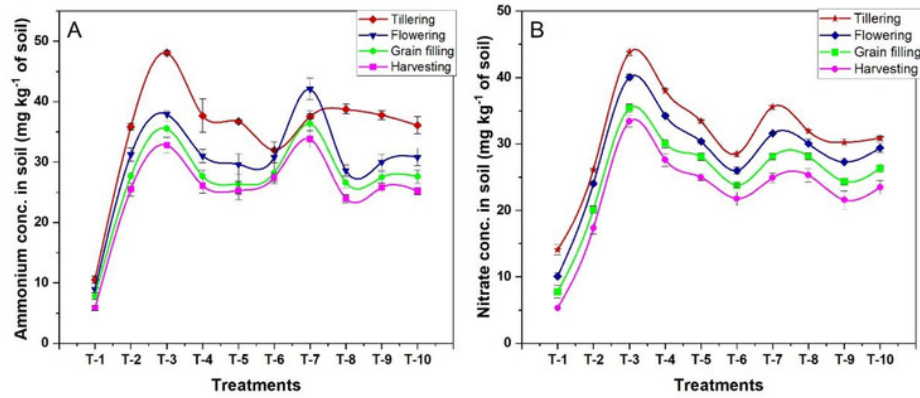


Figure 2. Effect of various organic and inorganic amendments on $\text{NH}_4^+\text{-N}$ and $\text{NO}_3\text{-N}$ concentration in soil

Figure 5

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

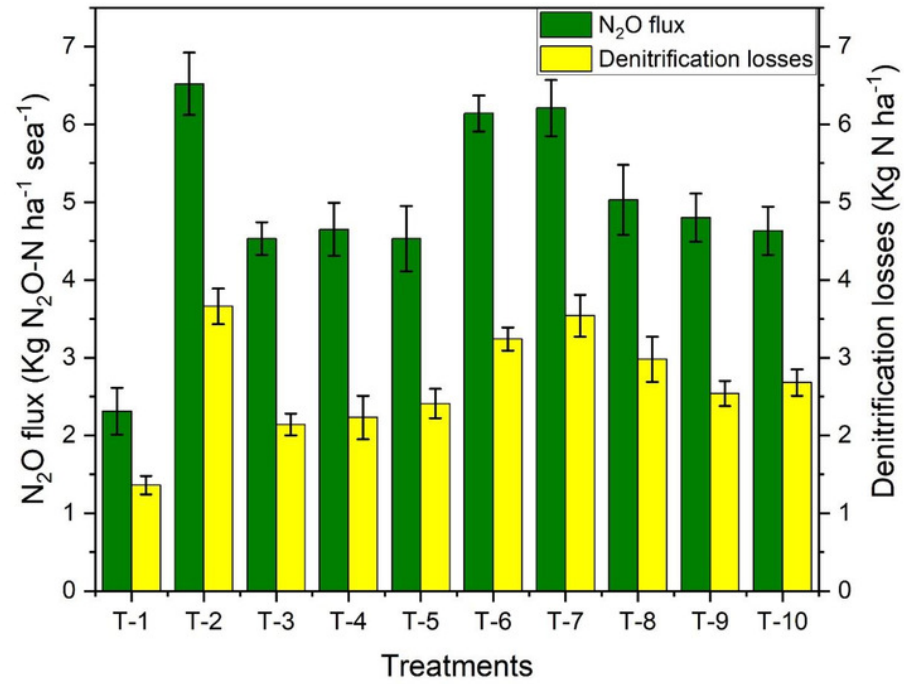


Figure 5. Effect of various organic and inorganic amendments on nitrous oxide flux and denitrification losses in soil

Figure 6

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

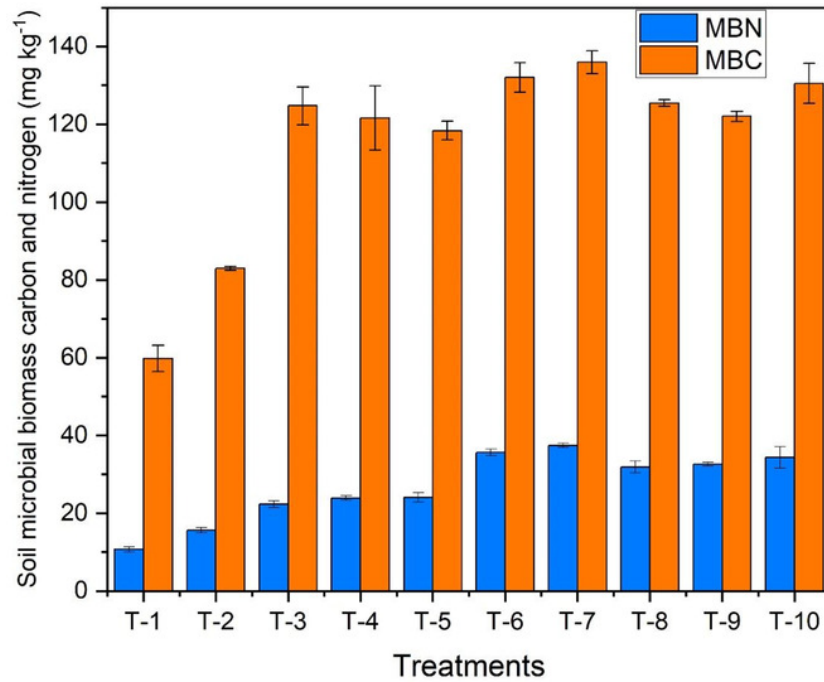


Figure 6. Effect of various organic and inorganic amendments on soil microbial biomass nitrogen and carbon (MBN and MBC) in soil

Figure 7

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

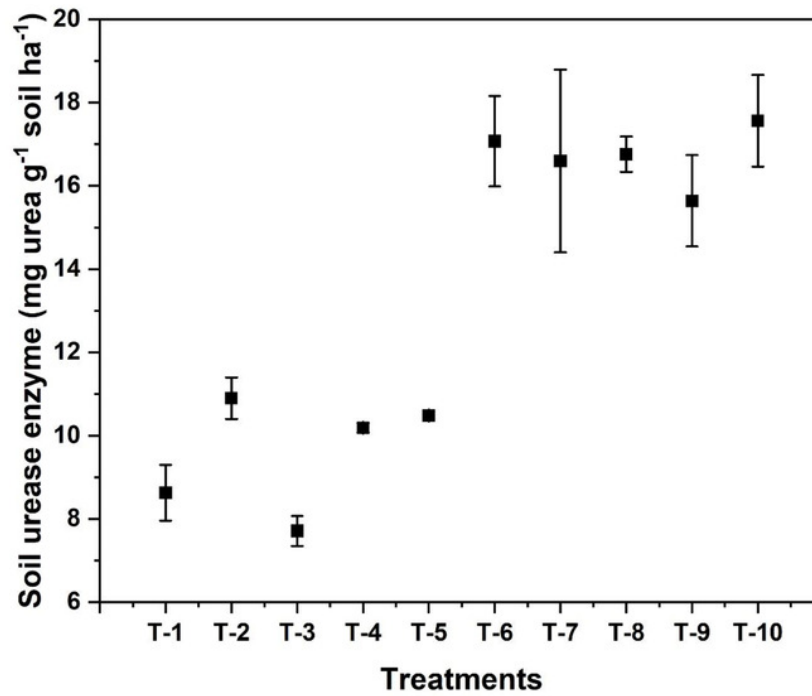


Figure 7. Effect of various organic and inorganic amendments on soil urease activity in wheat field