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 a 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 at about 20.4 kg ha^{-1} per season. Significant reduction in NH_3 volatilization losses were observed in T_3 by 40%, T_4 by 27%, and T_8 by 17% when compared to the control treatment. Soil urease activity was found to be decreased in plots 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 organic amendments is a feasible option to reduce NH_3 volatilization from wheat cultivation and improves NUE.

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], and Renu Singh[1]

[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

Background. This study focused on investigating 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 a Randomised 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 at about 20.4 kg ha^{-1} per season. Significant reductions in NH_3 volatilization losses were observed in T_3 by 40%, T_4 by 27%, and T_8 by 17% compared to the control treatment. Soil urease activity was found to be decreased in treatment T_3, T_4, and T_5 amended plots. The highest grain yield was observed in treatment T_7 with 5.09 t ha^{-1}, and straw yield was the maximum in treatment T_4 with 9.44 t ha^{-1}.

Conclusion. Based on the experimental data obtained, it can be concluded that organic amendments would be a feasible option to reduce NH_3 volatilization from wheat cultivation and improves NUE.

53

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 essential 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 a share of 24%. In the crop year 2020-21, India's wheat production increased from 109 Mt to 118 Mt to meet the growing

61 population's demand. However, in future, the wheat yield has to be increased by 1.5% per year
62 to satisfy the growing population's demand (*Grain and Feed Annual report, 2021*). Ammonia
63 volatilization is a significant cause of nitrogen depletion in agricultural soil worldwide,
64 contributing to low N fertilizer usage, crop production, and indirect nitrous oxide (N_2O)
65 emissions. Globally, the average NH_3 volatilization risks range from 0.9 to 64 % of the
66 applied N (a mean of 17.6 %) (*Pan et al., 2016*). Ammonia volatilization into the atmosphere
67 negatively affects agriculture, ecosystems, and human health.
68 Further, it also increases the loss of nitrogen for plant growth, thus increasing the cost of
69 cultivation (*Brink & Van Grinsven, 2011*). Secondary particulate matter (PM_{10}) is formed
70 when NH_3 reacts with other air contaminants such as sulfuric acid and nitric acid. It flies long
71 distances and remains in the air for several days, and causes respiratory diseases in humans
72 (*Bittman et al., 2014*).
73 A study conducted by Cao et al. (*2013*) found that the significant loss of N from applied
74 fertilizer was through NH_3 volatilization, which was estimated to be 10.0-19.5% of total N
75 loss. Further, they concluded that NH_3 volatilization might be the dominant pathway of N loss.
76 Ammonia volatilization favourably occurs in N fertilizers like urea and organic manure.
77 However, ammonia emission is undesirable as it removes N from the soil/plant system and
78 releases it into the atmosphere. Volatilized NH_3 is deposited back to the earth's surface mainly
79 through two processes which are i) Wet deposition through precipitation and ii) Dry
80 deposition when combined with particulate matter. This seriously impacts the environment as
81 it causes acidification of soil and water bodies and eutrophication of the natural ecosystem. It
82 also acts as an indirect source of N_2O, a potent greenhouse gas. In some instances, it also
83 directly affects plants under high concentrations and low temperatures. There are many other
84 ways to control N loss, like avoiding the application of urea under high-risk conditions, deep
85 placement, and using controlled-release N-fertilizers. But the more effective way to control is
86 by using organic and inorganic amendments; some of them are chemical inhibitors like N-(n-
87 butyl) thiophosphoric triamide (NBPT), Cyclohexyl phosphoric triamide (CHPT),
88 Ammonium thiosulphate (ATS), Hydroquinone (HQ), Calcium carbide (CaC_2).
89 Among these, NBPT is used globally, being the most effective in a market that has expanded
90 at 16 % per year over the last ten years. NBPT-treated urea reduces NH_3 loss by 53%
91 (*Cantarella et al., 2018*). Application of 12 kg N ha^{-1} HQ on an Alluvial soil, in conjunction

92   with 120 kg urea-N ha^{-1}, decreased N$_2$O emission by 5% in rice and 7% in wheat systems

93   when compared to the crops grown solely in the presence of 120 kg N ha^{-1} urea (*Modolo et*

94   *al., 2018*).

95   The slow-N-release coating technology is also suitable for consistent N supply to the plants

96   and reducing loss and contamination effects. We have explored various organic and inorganic

97   amendments to minimize NH$_3$ volatilization losses and enhance NUE and wheat crop

98   productivity. The application of N inhibitors along with urea has increased the average grain

99   yield by 6.8% (*Školníková et al., 2022*). Nitrification and urease inhibitors are being suggested

100   to decrease N losses and thus increase crop nitrogen usage efficiency (*Abalos et al.. 2014*).

101   Different mechanisms are involved in reducing NH$_3$ volatilization in that NBPT retards the

102   activity of the urease enzyme by competitive inhibition (*Fan et al., 2018*). Hydroquinone and

103   Calcium carbide is also effective in reducing urease activity. Applying HQ to the soil

104   inhibited urease enzyme activity and inhibited or enhanced the activity of other enzymes like

105   polyphenol oxidase, dehydrogenase, protease and phosphatase (*Wołejko et al., 2020*). The

106   CaC$_2$ has a negative impact on ammonia-oxidizing bacteria, which are present in the soil, and

107   also reduces the activity of dehydrogenase and nitrate reductase enzymes, thereby reducing

108   the N losses (*Mahmood et al., 2014*). Pongamia oil (karanjin) works as a nitrification inhibitor

109   by reducing the *Nitrosomonas* activity without affecting *Nitrobacter spp*. Activity. The

110   treatment with garlic extract, which is rich in the compound thiosulfinate, works on the

111   mechanism of competitive inhibition of urease enzyme as it contains an organosulphur group

112   (-S(O)-S) which is similar to urea which helps in inhibiting the hydrolysis process

113   (*Mathialagan et al., 2017*).

114   The world's population will surpass 9.7 billion by 2050, posing a significant obstacle to

115   achieving food sustainability. The projected increase in the world's population demands at

116   least a 70% increase in agriculture production in developed countries and 100% in developing

117   countries (*Mahmud et al., 2021*). Food security of the country and N-based global warming

118   and environmental degradation are interlinked (*Bilal & Aziz, 2022*). The use of urease

119   inhibitors in agricultural activities has already been investigated as one of the best methods for

120   ensuring adequate nutritional security (*Modolo et al., 2018*). N-(n-butyl) thiophosphoric

121   triamide treated urea lowers NH$_3$ loss by approximately 53%. The yield benefits from NBPT

122   application on an average of 6.0 % and ranges from 0.8 to 10.2 % depending on crop types

123 (*Cantarella et al., 2018*). The effectiveness of NBPT in reducing NH$_3$ loss is well known, but

124 there is still room for progress to increase the amount of inhibition and hence the retention of

125 NBPT-treated urea in the field. However, these inorganic amendments are not economically

126 viable, and most of them restricted their usage in the research areas due to high costs.

127 Upadhyay, Tewari & Patra (*2011*) investigated that these chemicals are harmful and inhibit

128 the growth of specific beneficial soil microorganisms that indirectly affects crop growth and

129 development. Therefore, the best option that stands before us is using organic amendments to

130 reduce NH$_3$ losses.

131 Organic amendments are eco-friendly, economically viable, and, most importantly,

132 biodegradable. Therefore, they act as promising N inhibitors. There are many plant-derived

133 inhibitors like Neem (*Azadirachta indica*) oil, Pongamia (*Pongamia glabra*) oil, Linseed

134 (*Linum usitatissimum*) oil, Garlic extract, and Mint (*Mentha spicata*) as N amendments in

135 inhibiting N losses. Thiosulfinates (TS) present in fresh garlic extract act as a bio-inhibitor of

136 urease enzyme activity and can be used as a potential urease inhibitor in agriculture.

137 Thiosulfinates have the potential to inhibit the urease enzyme, and it takes three hours and

138 thirty minutes to start acting on the urease enzyme after its' application (*Ramli et al., 2014*).

139 Applying coated urea fertilizers such as neem-coated urea and pine oleoresin-coated urea in a

140 vertisol reduced the NH$_3$ volatilization by 27.5% and 41.1%, respectively (*Jadon et al., 2018*).

141 But these organic amendments lack commercialization and efficient utilization. So this study

142 concentrates mainly on stressing the importance and comparative study of these organic and

143 inorganic amendments to decrease ammonia volatilization loss and improve increasing NUE

144 and productivity of Wheat (*Triticum aestivum* L.) crops.

145

Materials & Methods

147 **Details of the experimental site**

148 A field study was conducted at the research farm of the ICAR-Indian Agricultural Research

149 Institute, New Delhi, located at 280º 40 'N and 770º 12' E, at an altitude of 228.16 m above

150 mean sea level (sub-tropical and semi-arid region), during November 2020 to April 2021. The

151 Southwest monsoon contributed about 80% of rainfall, an average of 650 mm annually. The

152 pH of the soil was 8 (±0.1), with a sandy clay loam texture. The initial soil nutrients status,

153 when analyzed before sowing of seeds, were found as follows, the available N was low

154   (157±0.5 kg ha^{-1}), medium level of available P (13.5±0.2 kg ha^{-1}), and the available K

155   (196±0.8 kg ha^{-1}).

156

157   **Experimental design and management**

158   The experiment was conducted in a randomized block design (RBD) with 3 replications, and

159   each plot area was 12 m^2 (4 m×3 m). In all treatments recommended dose of nitrogen (RDN)

160   was applied except T$_1$ (control), where no RDN was used. The research was carried out

161   through growing wheat variety (HD 2967) with 10 treatments in respective plots, namely

162   labelled as T$_1$ (control), T$_2$ (RDN @ 150 kg ha^{-1}), T$_3$ (RDN + NBPT (N-(n-butyl)

163   thiophosphoric triamide) @ 0.5% w/w RDN), T$_4$ (RDN +HQ (Hydroquinone) @ 0.3% w/w of

164   RDN), T$_5$ (RDN + Calcium Carbide @ 1% w/w of RDN), T$_6$ (RDN + VAM (Vesicular

165   Arbuscular Mycorrhiza @ 10 kg ha^{-1}), T$_7$ (RDN + Azotobacter @ 50 g kg^{-1} seeds) T$_8$ (RDN +

166   Garlic powder @ 0.8% w/w of RDN), T$_9$ (RDN + Linseed oil @ 0.06% w/w of RDN), and

167   T$_{10}$ (RDN + Pongamia oil @ 0.06% w/w of RDN). Each dose was finalized based on the

168   current usage of these amendments by firms, which were applied in 2 splits (One at the time

169   of sowing and the second split application 30 days after sowing).

170   In order to study the effect of organic and inorganic amendments together, three inorganic

171   amendments, two bio-fertilizers, and three organic amendments were chosen based on

172   popularity and literature review. N-(n-butyl) thiophosphoric triamide (*Zanin et al., 2015*),

173   Hydroquinone (*Modolo et al.,2018*), Calcium Carbide (*Sakariyawo et al., 2020*), Garlic

174   powder (*Ramli et al., 2014*), Linseed oil, and Pongamia oil (*Majumdar, 2008*) were mixed

175   with urea (RDN) as per the dosage. Wheat seeds were treated with *Azotobater spp*. (as per

176   ICAR RABI Agro-Advisory for Farmers) and used for sowing in T$_7$. Vesicular Arbuscular

177   Mycorrhiza (as per ICAR RABI Agro-Advisory for Farmers) was applied directly into the soil

178   during sowing with an RDN. The recommended dose of fertilizers (RDF) for wheat was

179   applied as N: P$_2$O$_5$:K$_2$O (150:60:40 kg ha^{-1}). Urea, Single Super Phosphate (SSP), and

180   Muriate of Potash (MOP) were used as a source of N, P$_2$O$_5$, and K$_2$O, respectively.

181

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

183   Fresh soil samples were obtained from the 0-15 cm layer of soil at three separate sites from

184   each treatment using an 8 cm tube auger. Three soil samples were obtained from each

185   treatment during tillering, flowering, grain filling, and physiological maturity of the crop. The

186   total fresh soil samples were 30 in number and air-dried for 7 days, sieved through a 2 mm

187   screen, mixed, and placed in plastic bags for further analysis.

188

189   **Collection and analysis of NH$_3$**

190   The ammonia volatilization was monitored after fertilizer application for up to 10 days using a

191   forced air draft system method (*Bhaskar et al., 2022; Stumpe et al., 1984; Bremner, 1965*).

192   The closed chambers measuring 20 cm × 20 cm × 50 cm size made of 6 mm acrylic sheets

193   were placed in the field. The volatilized NH$_3$ gas from the soil surface under different

194   treatments was collected in a 2 % Boric acid solution containing a mixed indicator (methyl red

195   and bromocresol green). The air inside the chamber was collected into boric acid traps using a

196   vacuum pump having a flow rate of 3 L min^{-1}. The boric acid traps were changed every 24 hr.

197   The volatilized NH$_3$ was determined by the titration of boric acid solution with 0.02 N

198   sulphuric acid, and further calculations were done using the formula given below (Eq.1).

199   Volatilized ammonia (mg per m sq. per day) $= \dfrac{A * 0.00028 * 1000}{L * B}$ ………….. Eq.1

200

201   **Total Nitrogen and NH$_4^+$-N and NO$_3^-$-N analysis**

202   Total nitrogen content in soil was determined by the Kjeldahl method (*Kjeldahl, 1883*) during

203   the initial and after harvesting of crop growth. The NH$_4^+$-N and NO$_3^-$ -N were analyzed

204   through steam distillation (*Keeney and Bremner, 1965*) during all four crop growth stages

205   (Tillering, Flowering, Grain-filling, and Physiological maturity). The extract was prepared by

206   taking 10 g soils with 0.25 g activated charcoal and 50 mL KCl solution and kept for shaking

207   (30 min), then filtered with Whatman filter paper 1. From the same KCl extract, 10 mL each

208   was taken in two different distillation flasks, and 100 mL of distilled water was added to each

209   flask. In addition, 1g Devardas alloy was added for the case of NH$_4^+$-N estimation and NO$_3^-$-N

210   estimation 1 g magnesium oxide (MgO) was added and distilled separately, and these ions

211   were captured in 20 mL of 2% Boric acid and titrated against 0.02 N sulfuric acid. Further

212   calculations were done using equations 2 and 3 for NH$_4^+$-N estimation and NO$_3^-$-N

213   estimation, respectively.

214   Exchangeable ammonical N % in soil $= \dfrac{(Vs - Vb) \ * \ S \ * \ 0.014 \ * \ 100}{W} = Z1$…………Eq.2

215   Exchangeable NH$_4^+$-N (ppm) $= Z1 * 10^4$

216   Exchangeable nitrate N % in soil $= \frac{(Vs - Vb) \ * \ S \ * \ 0.014 \ * \ 100}{W} = Z2$ …………Eq. 3

217   Exchangeable NO_3^--N (ppm) = Z2 * 10^4

218   Where,

219   Vs denotes the volume of H_2SO_4 available for sample titration.

220   Vb denotes the volume of H_2SO_4 needed for blank titration.

221   S = H_2SO_4 power,

222   W = Weight of oven-dried soil used for analysis

223

224   Available Phosphorus (P) was analyzed using Olsens' estimation method (*Olsen et al., 1954*).

225   First, available P from the soil sample was extracted using 0.5 N $NaHCO_3$ solution buffer at

226   pH 8.5. Then available P in the extract was measured by an ascorbic acid method using a

227   spectrophotometer. Next, available potassium (K) in the soil was measured using an

228   ammonium acetate method (*Hanway & Heidel, 1952*), where available K was extracted by

229   shaking with neutral normal ammonium acetate for 5 min, and the K was determined using a

230   flame photometer. Finally, soil Organic Carbon was measured using Walkley and Black's

231   rapid titration method (*Walkley & Black, 1934*).

232

233   **Analysis of Nitrous oxide (N₂O) and other significant parameters**

234   Nitrous oxide flux was analyzed using the closed chamber method (*Herr et al., 2020*). In this

235   method, dark PVC boxes were installed, and the samples were drawn every 24 hrs in the

236   morning using syringes, evacuated into plastic vials, and analyzed chromatographically.

237   Denitrification losses were estimated by the denitrification enzyme assay method described by

238   Smith & Tiedje (*1979*).

239   Soil urease activity was analyzed at the 50% flowering stage, calorimetrically, by Bremner &

240   Douglas's (*1971*) method. Normalized Difference Vegetation Index (NDVI) was measured

241   using a green seeker (handheld crop sensor by Trimble) at the 50% flowering stage. Infrared

242   Gas Analyzer (LI-COR Model LI-6400X7 portable photosynthetic system) (IRGA) was used

243   to measure the photosynthetic rate and stomatal conductance. Soil Microbial Biomass Carbon

244   (MBC) and Soil Microbial Biomass Nitrogen (MBN) were determined by the chloroform

245   fumigation–extraction method described by Vance et al. (*1987*) and Brookes et al. (*1985*),

246   respectively. The N content in grains and straws was also measured using the Kjeldahl method

247   (*Kjeldahl, 1883*). After harvesting the crop, yield attributes were calculated from each plot.

248

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

250   The Nitrogen Use Efficiency can be defined as the ratio of outputs to inputs of nitrogen (*i.e.,*

251   NUE = N yield/N input).

252   Agronomic Efficiency of N (AEN) (kg ha^{-1}) = $\frac{GYF - GYN}{AFN}$..........Eq. 4

253   Production Efficiency of N (PEN) (kg kg^{-1}) = $\frac{GYF - GYN}{TUN - CUN}$..........Eq. 5

254   Apparent N Recovery (ANR) (%) = $\frac{TUN - CUN}{AFN}$×100.......... Eq. 6

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

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

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

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

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

260

261   **2.7 Statistical analysis**

262   The measurements obtained from the experimental work were analyzed by using OPSTAT.01

263   Software (O.P. Sheoran, Hisar, India) was used to calculate ANOVA, and means were

264   separated using Duncan Multiple Range Test (DMRT) at α=0.05. The data for each variable

265   was evaluated using variance protocol analysis for a Randomized block design, which was

266   checked using the "F" test for statistical significance (*Gomez & Gomez, 1984*). The Standard

267   Error of means (SEm) and Critical Difference (CD) parameters were calculated at a 5%

268   significance level.

269

270   # Results

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

272   The addition of organic and inorganic amendments in wheat crop have shown effective

273   controlled of ammonia volatilization losses during entire cropping season as shown in **Figure**

274   **1**. Results revealed that significant losses of N through NH$_3$ volatilization were observed in

275   the initial 5-6 days after N fertilizer application. The highest N volatilization losses were

276   observed in T_2 (20.4 kg ha^{-1} season^{-1}) and the lowest losses were found in T_1 (6.4 kg ha^{-1}

277   season^{-1}). T_3 performed well in reducing the NH_3 losses by 40% as compared to T_2. NH_3

278   losses in T_3, T_4 and T_8 were recorded 12.4, 14.6, and 16.6 kg ha^{-1} season^{-1}, respectively

279   (Figure 2). Duncan Multiple Range Test revealed that T_3 (NBPT) and T_4 (HQ) effectively

280   reduced the NH_3 losses. T_5 (CaC$_2$) and T_8 (Garlic powder) showed no significant difference

281   among their means. However, among plant-based amendments, T_8 with garlic powder-treated

282   urea showed better results by reducing NH_3 volatilization losses by 17% compared to only

283   RDF-treated urea (T_2). Other treatments were not so effective in reducing the NH_3 losses.

284

285   **Initial and final soil nutrient status**

286   Available nitrogen content in soil after wheat crop harvest was found highest in treatment T_7

287   (*i.e.,* Azotobacter treated plot) which was 195 kg ha^{-1}. Detailed observations on Available N

288   content for different treatments is given in Table 1. The highest SOC was observed in T_7,

289   treated with Azotobacter spp., *i.e.,* 0.63%. It significantly increased the SOC content

290   compared to the initial SOC (0.42%) before sowing the wheat crop, as shown in Figure 3. The

291   highest level of available P was observed in T_6 treated with VAM, i.e., 30.4 kg ha^{-1}, which is

292   more than double the initial value of available P, as shown in Table 1. The initial level of

293   available K was 196 kg ha^{-1}, while in the final analysis after harvesting the crop, it increased

294   to 229 kg ha^{-1} in T_{10}, as depicted in Table 1.

295

296   **Ammonical N (NH_4^+-N) and Nitrate (NO_3^-N) in the soil**

297   The available nitrogen forms, mostly NH_4^+-N and NO_3^--N in the soil, decreased from tillering

298   to the grain filling stage (Figure 4.). The mean quantities of NH_4^+-N in the soil at various crop

299   growth stages (tillering, flowering, grain filling, and physiological maturity stage) were 78.6

300   kg ha^{-1}, 65.6 kg ha^{-1}, 57.9 kg ha^{-1}, and 53.5 kg ha^{-1}, respectively. The mean of NO_3^--N in the

301   soil at all four growth stages was 70 kg ha^{-1}, 63.4 kg ha^{-1}, 56.5 kg ha^{-1}, and 51.1 kg ha^{-1},

302   respectively. During the tillering stage, the highest NH_4^+-N (108 kg ha^{-1}) was observed in T_3,

303   *i.e.,* treatment with NBPT amended urea, and the highest NO_3^--N (58.5 kg ha^{-1}) was found in

304   RDN treated plot (T_2). In all the stages of plant growth, T_3 showed significantly higher values

305   of NH_4^+-N, which was 47% higher than T_2. Duncan Multiple Range Test also showed that the

306   mean of treatments T_4, T_7, and T_8 was also significantly at par for the available NH_4^+-N

307   content in the soil, and in the case of NO_3^--N availability, the mean of treatments T_4, T_7, and

308   T_5 also showed significant difference during all the stages of the wheat crop growth.

309

310   **Nitrous oxide flux and denitrification losses from the soil**

311   Nitrous oxide emission is directly related to the amount of N fertilizer available in the soil.

312   The highest cumulative N_2O flux was observed in T_2 (RDF) plots with 6.52 kg N_2O-N ha^{-1}

313   season^{-1}. There were no significant differences between T_3, T_4, and T_5 in the case of N_2O

314   emission reduction (Figure 3). But all of them were significant when compared with T_2.

315   Among plant-based inhibitor treatments, T_8, having urea amended with garlic powder, showed

316   effective results. On the other hand, the highest denitrification losses were observed in T_2

317   (3.66 kg-N ha^{-1}) (Figure 5). N-(n-butyl) thiophosphoric triamide treated urea was

318   comparatively effective by reducing the denitrification losses by 35 %.

319

320   **Soil microbial biomass carbon and nitrogen in the soil**

321   Soil biomass carbon and soil biomass nitrogen (found within living organisms like fungi and

322   bacteria) were observed at the 50% flowering stage of the crop. The highest MBN content was

323   reported in T_7, *i.e., Azotobacter* spp. treated plot with 37 mg kg^{-1}, followed by T_6, having

324   VAM treated plot. The highest MBC (136 mg kg^{-1}) was observed in T_7, *Azotobacter* spp.

325   treated plot was 63.9% more than T_2 (Figure 6).

326

327   **Soil urease enzyme activity in the soil**

328   As shown in Figure 7, under the 50% flowering stage in T_2, i.e., only RDN treated plot

329   showed the highest urease activity (18.9 mg urea g^{-1} soil h^{-1}) and the lowest urease activity

330   (7.71 mg urea g^{-1} soil h^{-1}) was observed in T_3 treatment containing NBPT which was indicated

331   by the effectiveness of urease inhibitor.

332

333   **Physiological parameters of wheat crop**

334   Adding organic and inorganic amendments to the soil has significantly influenced the physiological

335   parameters. The range of photosynthesis rate varied from 15.7 μ mol m^{-2} s^{-1} to 23.8 μ mol m^{-2} s^{-1},

336   which was recorded during the 50% flowering stage. The highest photosynthetic rate was observed

337   in T_7 with 23.8 μ mol m^{-2} s^{-1}, followed by T_3 and T_6 (23.3 μ mol m^{-2} s^{-1} and 22.9 μ mol m^{-2} s^{-1}).

338   The range of chlorophyll content varied from 0.66 to 0.77, recorded during the 50% flowering

339   stage. The highest chlorophyll content was observed in both T_4 and T_7, with 0.77, followed by

340   T_6 and T_{10}, showing 0.76. The highest LAI was observed in T_7 with 4.59, followed by T_3 and

341   T_8, which recorded the same LAI, i.e., 4.16. The lowest reading was recorded in treatment T_1,

342   *i.e.,* 1.85. The highest stomatal conductance was observed in T_7 with 0.48 m mol m^{-2} s^{-1},

343   followed by T_3 and T_8 (0.47 m mol m^{-2} s^{-1} and 0.43 m mol m^{-2} s^{-1}), respectively.

344

345   **Nitrogen uptake by wheat**

346   The data about the nitrogen content analyzed in wheat grain and straw samples after harvest

347   depicted the positive effect of both organic and inorganic amendments. The *Azotobacter spp.*

348   treated plot (T_7) showed 38.7% higher nitrogen content in grains when compared to T_2

349   treatments, as shown in Table 1. The highest nitrogen content in straw was observed in T_3

350   (NBPT), almost double the straw nitrogen content of the T_1 (control) plot.

351   **Wheat yield and nitrogen use efficiency**

352   The data obtained in grain and straw yield showed significant improvement in yields due to

353   the addition of organic and inorganic amendments. The highest grain yield was observed in

354   T_3, *i.e.,* treatment with NBPT amended urea with 5.09 t ha^{-1}, and straw yield was in T_4, *i.e.,*

355   treatment with hydroquinone amended urea (Table 2). However, in the case of straw yield, all

356   the treatment means were not significantly different at $p=0.05$. The other yield attributes like

357   biological yield, number of tillers per m^{-2}, number of spikes per m^{-2}, number of grains per

358   spike, and harvest index were not significantly influenced by amendments addition ($p=0.05$).

359   However, the test weight of the wheat grain of treatment T_8 (39.8 g) was significant among all

360   the treatments.

361   As nitrogen content in the grains has increased on amending urea with organic and inorganic

362   amendments, NUE was calculated in terms of Agronomic Efficiency of N (AEN), Production

363   Efficiency of N (PEN), and Apparent N Recovery (ANR) using Equations 4,5, and 6. The

364   highest AEN was found in T_3, with 12.9 kg ha^{-1}, and the lowest was in T_2, with 9.05 kg ha^{-1}

365   (Table 1). The highest PEN was observed in T_2 with 25.8 kg kg^{-1}, and the lowest was in T_7

366   (15.4 kg kg^{-1}). The average value of PEN was 17.9 kg kg^{-1}. The highest ARN (81 %) was seen

367   in T_7, and the lowest was in T_2 (35 %). The mean value of ANR was observed to be 49.6%.

368

Discussion

Our experimental findings in the case of NH_3 volatilization losses depicted that T_3 outperformed all other treatments because of competitive inhibition of urease enzyme activity by NBPT in the soil, which has a significant 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 like 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, thereby reducing the N losses, even though many studies have been taken on the case of NBPT. There is still a need to improve the duration of inhibition of urease activity, the shelf life of urea fertilizer coated with NBPT, and the economic viability of these compounds.

Agriculture alone contributes 80-90% of NH_3 emissions globally, mainly through volatilization from livestock and synthetic nitrogen fertilizer (*Xu et al., 2019*). It acts as a pollutant influencing the biosphere through haze formation and soil acidification. N_2O, a potent greenhouse gas, is also of great concern, contributes to 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 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*).

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*), reported a decrease of 31.6% N, which used to be lost if not applied with thiosulfinates, and concluded it was an upcoming natural urease inhibitor.

399 The final analysis of TNC, available N, P, and K, reported an increase in all these nutrient

400 contents to the initial analysis, which helps the next sown crop in nutrition. T_7 treated with

401 Azotobacter *spp.* performed well in increasing N and K content in the soil, and T_6 was treated

402 with VAM to increase P content in the soil. An increase in the N content of the soil could be

403 due to adding these bio-fertilizers as amendments that significantly increased residual N and

404 reduced the N fertilizer application for the next crop, which will be sown in that plots.

405 Vesicular Arbuscular Mycorrhiza improves P mobilization; hence uptake by wheat crops and P

406 content in soil increases. The addition of bio-fertilizers as an amendments source to reduce N

407 losses has improved the soil SOC.

408 The highest SOC was observed in T_7, which was treated with Azotobacter spp. i.e., 0.63%. It

409 has doubled the SOC content compared to the initial SOC before sowing the wheat crop, i.e.,

410 0.42%. A similar effect of these bio-fertilizers in increasing total nitrogen content was observed

411 in a study on Bio-fertilizer affecting structural dynamics, function, and network patterns of the

412 sugarcane rhizospheric microbiota by Liu et al. (*2021*). Kader et al. (*2002*) also found similar

413 results while working on the effect of Azotobacter spp. Inoculants on wheat yield and nitrogen

414 uptake. This result is supported by Suri et al. (*2011*) while working on the influence of VAM

415 and applied P on root colonization in wheat.

416

417 The available N forms, mostly NH_4^+-N and NO_3^--N, decreased from the tillering stage to the

418 grain-filling stage due to uptake and losses. During the tillering stage, the highest NH_4^+-N

419 and NO_3^--N was observed in T_3, i.e., treatment with NBPT amended urea due to a slowdown

420 of urease activity, thereby enhancing the accumulation of NH_4^+ and NO_3^- ions, and this

421 increased the plant uptake. The urease activity was slowed down when NBPT-amended urea

422 was applied to the soil, thus, enhancing the accumulation of NH_4^+ ions and increasing the

423 plant uptake of NH_4^+ and NO_3^- ions. During N mineralization from the applied N fertilizers,

424 most of the N remains as NH_4^+ ions, then rapidly converting into NO_3^- due to the inhibitory

425 effect of NBPT. That also helps in reducing NO_3^- leaching. A similar result was found by

426 Dhakar et al. (*2015*) while working on the impact of nitrification inhibitors and various

427 nitrogen sources on soil nitrogen distribution in Kinnow orchards.

428 As NH_4^+ availability was initially low for the conversion into N_2O, the losses were low in the

429 initial 7 days due to the application of these inhibitors. The application of urease inhibitors as

430   amendments and urea regulates the concentration of NH_4^+ and NO_3^- in the soil, thereby

431   commanding NH_3 and N_2O emissions (*Ding et al., 2015*). Denitrification losses depend on

432   soil moisture, soil temperature, and N-ions availability. Even though NBPT has no direct

433   effect on lowering denitrification losses, it was observed that it had influenced the reduction

434   of the emission of N_2O (*Cassim et al., 2021*). The reduction in the availability of NO_3^- has

435   controlled the processes of denitrification and N_2O emission, mainly in the summer. The

436   primary controller of denitrification processes in winter wheat is soil aeration and temperature

437   (*Aulakh et al., 2001*).

438

439   Amendments like bio-fertilisers *Azotobacter spp*. and VAM positively affect soil biomass

440   carbon and soil biomass nitrogen. These microbes help decompose organic matter and release

441   the essential nutrients for plant uptake, increasing the MBC and MBN of the soil. Similar

442   results were observed by Faujdar (*2011*) while working on the effect of FYM, bio-fertilizers,

443   and zinc on nutrient transformations, soil properties, and yield of maize, and their residual

444   effect on wheat.

445

446   The soil urease enzyme was suppressed by N inhibitors by a competitive inhibition

447   mechanism, thereby reducing or slowing down the enzyme's activity. Rapid losses in N are

448   mainly due to urea hydrolysis by the urease enzyme, which drastically increases pH and NH_4^+

449   ion concentration in the soil (*Liu et al., 2018*). So, this rise in pH and ion concentration

450   increases NH_3 volatilization losses. In the entire growth period of wheat, the urease activity in

451   the soil was always at a peak, especially during tillering stage; as it grew to maturity, its'

452   activity dropped. Similar results were reported by Fu et al. (*2019*) while working on the

453   effects of urease and nitrification inhibitors on the soil.

454

455   The physiological characteristics of wheat analyzed (photosynthesis rate, chlorophyll

456   content, LAI, and stomatal conductance) were also significantly influenced by organic and

457   inorganic amendments. In this study, photosynthesis rate, chlorophyll content, LAI, and

458   stomatal conductance were slightly increased in T_7 treatment with azotobacter because of an

459   increase in nitrogen availability, which positively affects these physiological parameters.

460   Furthermore, followed by Azotobacter treated plot, NBPT treated plot showed significant

461   improvement in all these parameters.

462

463   The increase in the N content in wheat straw and grain might be due to improved nitrogen

464   availability due to a reduced N loss and better NUE. As nitrogen is the most limiting nutrient

465   in the growth and development of the wheat crop, the better uptake of the nutrient N will

466   affect plant growth which will help to attain global food security by increasing the wheat

467   yield by 1.5% per year. These results were similar to those obtained by Singh et al. (*2018*)

468   while working on certain microorganisms like Bacillus spp. and Azotobacter spp. Mukhtar,

469   Bashir & Nawaz (*2018*) found that microorganisms enhance crop growth by making nutrients

470   available by fixing nitrogen and phosphate solubilization. In addition, *azotobacter spp.* can

471   produce a few metabolites like phytohormones and exopolysaccharides, which help crops

472   absorb nutrients and develop roots (Hindersah *et al., 2020*). Both are inorganic amendments

473   that have effectively improved the plant's nutrient availability, thereby increasing yield. These

474   findings were similar to those of Kumar et al. (*2015*), who reported an increase in grain and

475   biological yield by 22.6% and 17.4%, respectively. It was also reported by Galindo et al.

476   (*2020*) that NBPT has some effect on metabolic pathways in decreasing urease enzyme

477   activity hence increasing the NUE.

478   Furthermore, slowing down nutrient release has improved the period of availability of N and

479   increased the crop's N uptake (*Liu et al., 2020*). These positive effects, in turn, enhanced

480   wheat's growth, development, and yield. However, in reducing NH_3 volatilization NBPT, HQ

481   amendments performed much better than other amendments, but these amendments have

482   some adverse effects on soil microorganism activity as per the studies by Upadhyay, Tewari

483   & Patra (*2011*).

484

485   **Conclusions**

486   The current study concludes that both organic and inorganic amendments significantly reduce

487   NH_3 losses, thus increasing available soil N and enhancing the NUE of the crops. Treatment

488   containing NBPT reduced the losses by 40% compared to only RDN without any

489   amendments. These results may be due to strong inhibition of urease activity by NBPT in the

490   soil. Among plant-based amendments, garlic powder-treated urea showed better results in

491   decreasing NH$_3$ volatilization losses by 17% compared to only RDN-treated urea. These

492   positive effects, in turn, enhanced the growth, development, and yields of wheat. Even though

493   inorganic amendments performed much better than organic amendments in reducing NH$_3$

494   volatilization, opting for organic amendments gives way to sustainable agriculture. Thus, the

495   focus should be more on organic than inorganic amendments to reduce NH$_3$ emissions from

496   agricultural fields.

497

498

499   **References**

500   Abalos D, Jeffery S, Sanz-Cobena A, Guardia G, Vallejo A. 2014. Meta-analysis of the effect

501   of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency.

502   *Agriculture, Ecosystems & Environment* 189: 136-144.

503   Affendi NMN, Mansor N, Samiri SS. 2020. Addition of chemical and natural urease inhibitors

504   reduces ammonia and nitrous oxide losses. *Journal of Soil Science and Plant Nutrition* 20(1):

505   253-258.

506   Aulakh MS, Khera TS, Doran JW, Bronson KF. 2001. Denitrification, N$_2$O and CO$_2$ fluxes in

507   rice-wheat cropping system as affected by crop residues, fertilizer N and legume green

508   manure. *Biology and Fertility of Soils* 34(6): 375-389.

509   Bhaskar KA, Al-Hashimi A, Meena M, Meena VS, Langyan S, Shrivastava M, Sayyed

510   RZ, El-Enshasy HA, Almunqedhi BMA, Singh R. 2022. Conservation agricultural practices

511   for minimizing ammonia volatilization and maximizing wheat productivity. *Environmental*

512   *Science and Pollution Research* 29: 9792–9804 DOI: https://doi.org/10.1007/s11356-021-

513   16370-4.

514   Bilal HM, Aziz T. 2022. Nitrogen use efficiency in crop production: issues and challenges in

515   South Asia. Nitrogen Assessment. 127-148.

516   Bittman S, Dedina M, Howard CM, Oenema O, Sutton MA. 2014. Options for ammonia

517   mitigation. Guidance from the UNECE Task Force on Reactive Nitrogen, chapter 8, Centre

518   for Ecology and Hydrology, Edinburgh, UK.

519   Bremner JT. 1965. Inorganic forms of nitrogen. Methods of Soil Analysis, *Part 2 Chemical*

520   *and Microbiological Properties* 9: 1179-1237.

521   Bremner JM, Douglas LA. 1971. Inhibition of urease activity in soils. *Soil Biology &*

522   *Biochemistry* 3(4): 297-307.

523   Bremner JM, Keeney DR. 1965. Steam distillation methods for determination of ammonium,

524   nitrate and nitrite. *Analytica Chimica Acta* 32: 485-495.

525   Brink C, Van Grinsven H. 2011. Cost and benefits of nitrogen in the environment. The

526   European Nitrogen Assessment, chapter 22, Cambridge University Press.

527   Brookes PC, Landman A, Pruden G, Jenkinson DS. 1985. Chloroform fumigation and the

528   release of soil nitrogen: a rapid direct extraction method to measure microbial biomass

529   nitrogen in soil. *Soil Biology & Biochemistry* 17(6): 837-842.

530   Cameron KC, Di H J, Moir JL. 2013. Nitrogen losses from the soil/plant system, a

531   review. *Annals of Applied Biology* 162(2): 145-173.

532   Cantarella H, Otto R, Soares JR, de Brito Silva AG. 2018. Agronomic efficiency of NBPT as

533   a urease inhibitor, A review. *Journal of Advanced Research* 13:19-27.

534   Cao Y, Tian Y, Yin B, Zhu Z. 2013. Assessment of ammonia volatilization from paddy fields

535   under crop management practices aimed to increase grain yield and N efficiency. *Field Crops*

536   *Research* 147: 23-31.

537   Cassim BMAR, Kachinski WD, Besen MR, Coneglian CF, Macon CR, Paschoeto GF, Batista

538   MA. 2021. Duromide increase NBPT efficiency in reducing ammonia volatilization loss from

539   urea. *Revista Brasileira de Ciencia Avicola* 45.

540   Dhakar MK, Singh AK, Patel VB, Singh SK, Datta SP, Kumar R, Khanna M. 2015. Effect of

541   different nitrogen sources and nitrification inhibitors on soil nitrogen distribution in Kinnow

542   orchard. *Indian Journal of Horticulture* 72(2): 178-182.

543   Ding WX, Chen ZM, Yu HY, Luo JF, Yoo GY, Xiang J, Yuan JJ. 2015. Nitrous oxide

544   emission and nitrogen use efficiency in response to nitrophosphate, N-(n-butyl)

545   thiophosphoric triamide and dicyandiamide of a wheat cultivated soil under sub-humid

546   monsoon conditions. *Biogeosciences* 12(3), 803-815.

547   Eduardo LC, Silva DRG, Faquin V, de Almeida Gonçalves B, Cancellier LL, Spehar CR.

548   2016. Ammonia volatilization from enhance defficiency urea on no-till maize in Brazilian

549   cerrado with improved soil fertility. *Ciencia e Agrotecnologia* 40(2):133–144.

550   Fan X, Yin C, Yan G, Cui P, Shen Q, Wang Q, Chen H, Zhang N, Ye M, Zhao Y and Li T.

551   2018. The contrasting effects of N-(n-butyl) thiophosphoric triamide (NBPT) on N_2O

552   emissions in arable soils differing in pH are underlain by complex microbial

553   mechanisms. *Science of the Total Environment* 642:155-167.

554   Faujdar RS. 2011. Effect of FYM, biofertilizers and zinc on nutrient transformations, soil

555   properties and yield of maize (*Zea mays* L.) and their residual effect on wheat (*Triticum*

556   *aestivum* L.) on typic haplustept, MPUAT, Udaipur.

557   Fu Q, Abadie M, Blaud A, Carswell A, Misselbrook TH, Clark IM, Hirsch PR. 2020. Effects

558   of urease and nitrification inhibitors on soil N, nitrifier abundance and activity in a sandy

559   loam soil. *Biology and Fertility of Soils* 56(2): 185-194.

560   Galindo FS, Teixeira Filho MCM, Buzetti S, Pagliari PH, Santini JMK. 2020. Can NBPT

561   urease inhibitor in combination with Azospirillum brasilense inoculation improve wheat

562   development? *Nutrient Cycling in Agroecosystems* 1-13.

563   Gomez K, Gomez AA. 1984. Statistical procedure for agriculture research. Wily and Sone.

564   Grain and Feed Annual report. 2021. United States Department of Agriculture. 2-3. DOI:

565   https://apps.fas.usda.gov.

566   Hanway JJ, Heidel H. 1952. Soil analysis methods as used in Iowa state college soil testing

567   laboratory. *Iowa Agriculture*. 57: 1-31.

568   Herr C, Mannheim T, Müller T, Ruser R. 2020. Effect of nitrification inhibitors on N_2O

569   emissions after cattle slurry application. *Agronomy* 10(8): 1174.

570   Hindersah R, Setiawati MR, Asmiran P, Fitriatin BN. 2020. Formulation of Bacillus and

571   Azotobacter consortia in liquid cultures, preliminary research on microbes-coated

572   urea. *International Journal of Agriculture System* 8(1): 1-10.

573   ICAR RABI Agro-Advisory for Farmers-2021-22:https://icar.org.in/content/icar-rabi-agro-

574   advisory-farmers

575   Jain N, Singh GP, Yadav R, Pandey R, Ramya P, Shine MB, Prabhu KV. 2014. Root trait

576   characteristics and genotypic response in wheat under different water regimes. *Cereal*

577   *Research Communications* 42(3): 426-438.

578   Jadon P, Selladurai R, Yadav SS, Coumar MV, Dotaniya ML, Singh AK & Kundu S. 2018.

579   Volatilization and leaching losses of nitrogen from different coated urea fertilizers. *Journal of*

580   *soil science and plant nutrition* 18(4): 1036-1047.

581   Kader MA, Mian MH, Hoque MS. 2002. Effects of Azotobacter inoculant on the yield and

582   nitrogen uptake by wheat. *Journal of Biological Sciences* 2(4): 259-261.

583 Kjeldahl C. 1883. A new method for the determination of nitrogen in organic matter.

584 *Analytical Chemistry* 22: 366.

585 Kumar B, Dhar S, Vyas AK, Paramesh V. 2015. Impact of irrigation schedules and nutrient

586 management on growth, yield and root traits of wheat (*Triticum aestivum*) varieties. *Indian*

587 *Journal of Agronomy* 60(1): 87-91.

588 Lasisi AA, Akinremi OO, Kumaragamage D. 2019. Efficacy of a new N-(n-butyl)

589 thiophosphoric triamide formulation in reducing ammonia volatilization from urea-based

590 fertilizers. *Canadian Journal of Soil Science* 99(4): 395-405.

591 Leghari SJ, Wahocho NA, Laghari GM, Hafeez Laghari A, MustafaBhabhan G,

592 HussainTalpur K, Lashari AA. 2016. Role of nitrogen for plant growth and development, A

593 review. *Advances in Environmental Biology* 10(9): 209-219.

594 Li Q, Yang A, Wang Z, Roelcke M, Chen X, Zhang F, Liu X. 2015. Effect of a new urease

595 inhibitor on ammonia volatilization and nitrogen utilization in wheat in north and northwest

596 China. *Field Crops Research* 175: 96-105.

597 Liu G, Yang Z, Du J, He A, Yang H, Xue G, Yu C, Zhang Y. 2020. Adding NBPT to urea

598 increases N use efficiency of maize and decreases the abundance of N-cycling soil microbes

599 under reduced fertilizer-N rate on the North China Plain, *Plos one,* 15(10): pp. e0240925

600 Liu Q, Pang Z, Yang Z, Nyumah F, Hu C, Lin W, Yuan Z. 2021. Bio-fertilizer Affects

601 Structural Dynamics, Function, and Network Patterns of the Sugarcane Rhizospheric

602 Microbiota. *Microbial Ecology* 1-17.

603 Liu Y, Dai Q, Jin X, Dong X, Peng J, Wu M, Liang N, Pan B, Xing B. 2018. Negative

604 impacts of biochars on urease activity: high pH, heavy metals, polycyclic aromatic

605 hydrocarbons, or free radicals? *Environmental Science & Technology*   52(21): 12740-12747.

606 Mahmood R, Ali A, Yaseen M, Ahmad M. 2014. Role of encapsulated calcium carbide in

607 nitrogen economy, growth and yield of crops: a review. *Journal of Animal and Plant*

608 *Sciences* 24(2).

609 Mahmud K, Panday D, Mergoum A, Missaoui A. 2021. Nitrogen Losses and Potential

610 Mitigation Strategies for a Sustainable Agroecosystem. *Sustainability* 13:2400. DOI:

611 https://doi.org/ 10.3390/su13042400

612 Majumdar D. 2008. Unexploited botanical nitrification inhibitors prepared from Karanja

613 plant. *Indian Journal of Natural Products and Resources* 7: 58-67.

614   Mathialagan R, Mansor N, Al-Khateeb B, Mohamad MH, Shamsuddin MR. 2017. Evaluation

615   of allicin as soil urease inhibitor. *Procedia Engineering.* 184: 449-459.

616   Modolo LV., da-Silva CJ, Brandão DS, Chaves IS. 2018. A minireview on what we have

617   learned about urease inhibitors of agricultural interest since mid-2000s. *Journal of Advanced*

618   *Research* 13: 29-37.

619   Mukhtar H, Bashir H, Nawaz A. 2018. Optimization of growth conditions for Azotobacter

620   species and their use as biofertilizer. *Journal of Bacteriology and Mycology* 6(5): 274-278.

621   Olsen SR. 1954. Estimation of available phosphorus in soils by extraction with sodium

622   bicarbonate (No. 939). US Department of Agriculture.

623   Pan B, Lam SK, Mosier A, Luo Y, Chen D. 2016. Ammonia volatilization from synthetic

624   fertilizers and its mitigation strategies: A global synthesis. *Agriculture, Ecosystems &*

625   *Environment* 232: 283-289.

626   Ramli NK, Majeed Z, Shuib A, Mansor N, Man Z. 2014. The potential of thiosulfinates in

627   garlic extract as urease bioinhibitor. *In Applied Mechanics and Materials* 625:796-799. Trans

628   Tech Publications Ltd.

629   Sakariyawo OS, Oyeledun KO, Adeyemi NO, Atayese MO. 2020. Nitrogen use efficiency and

630   performance of maize (*Zea mays* L.) cultivars as influenced by calcium carbide and inorganic

631   nitrogen application rates in a derived savanna. *Journal of Plant Nutrition* 43(6): 784-797.

632   Sheoran OP, Tonk DS, Kaushik LS, Hasija RC and Pannu RS .1998. Statistical Software

633   Package for Agricultural Research Workers. Recent Advances in information theory,

634   Statistics & Computer Applications by D.S. Hooda & R.C. Hasija Department of Mathematics

635   Statistics, CCS HAU, Hisar:139-143

636   Singh G, Kumar S, Sidhu GS, Kaur R. 2018. Effect of integrated nutrient management on

637   yield of wheat (*Triticum aestivum* L.) under irrigated conditions. *International journal of*

638   *chemical studies* 6:904-907.

639   Školníková M, Škarpa P, Ryant P, Kozáková Z, Antošovský J. 2022. Response of Winter

640   Wheat (*Triticum aestivum* L.) to Fertilizers with Nitrogen-Transformation Inhibitors and

641   Timing of Their Application under Field Conditions. *Agronomy* 12(1): 223.

642   Smith MS, JM Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. *Soil*

643   *Biology & Biochemistry* 2: 262-267.

644 Stumpe JM, Vlek PLG, Lindsay WL. 1984. Ammonia volatilization from urea and urea
645 phosphates in calcareous soils. *Soil Science Society of America Journal* 48(4): 921-992.
646 Suri VK, Choudhary AK, Chander G, Verma TS. 2011. Influence of vesicular-arbuscular
647 mycorrhizal fungi and applied phosphorus on root colonization in wheat and plant nutrient
648 dynamics in a phosphorus-deficient acid alfisol of Western Himalayas. *Communications in*
649 *Soil Science and Plant Analysis* 42(10): 1177-1186.
650 Upadhyay RK, Tewari SK, Patra DD. 2011. Natural nitrification inhibitors for higher nitrogen
651 use efficiency, crop yield, and for curtailing global warming. *Journal of Tropical Agriculture*
652 49: 19-24
653 Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil
654 microbial biomass. *Soil Biology & Biochemistry* 19(6): 703-707.
655 Walkley A, Black IA. 1934. An examination of the Degtjareff method for determining soil
656 organic matter and a proposed modification of the chromic acid titration method. *Soil Science*
657 37(1): 29-38.
658 Wołejko E, Jabłońska-Trypuć A, Wydro U, Butarewicz A, and Łozowicka B. 2020. Soil
659 biological activity as an indicator of soil pollution with pesticides–a review. *Applied Soil*
660 *Ecology* 147: 103356.
661 Xu R, Hanqin Tian, Shufen Pan, Stephen A, Yucheng Feng, William D, Jian Chen and Jia
662 Yang. 2019. Global ammonia emissions from synthetic nitrogen fertilizer applications in
663 agricultural systems: Empirical and process-based estimates and uncertainty. *Global Change*
664 *Biology* 25:314–326, https://doi.org/10.1111/gcb.14499.
665 Zanin L, Tomasi N, Zamboni A, Varanini Z, Pinton R. 2015. The urease inhibitor NBPT
666 negatively affects DUR3-mediated uptake and assimilation of urea in maize roots. *Frontiers*
667 *in plant science* 6: 1007.
668
669

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	-	-	-

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)**

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

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   (α=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.)

Figure 1

Figure 1. Temporal graph of ammonia flux and effect of organic and inorganic amendments on ammonia flux.
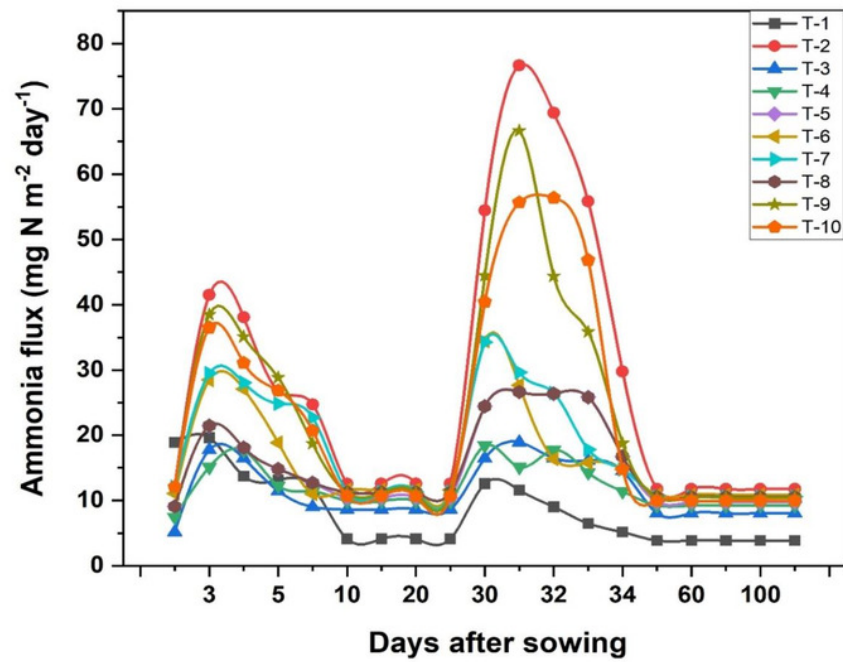
Figure 2

Figure 2. Effect of various organic and inorganic amendments on ammonia volatilization losses from the soil
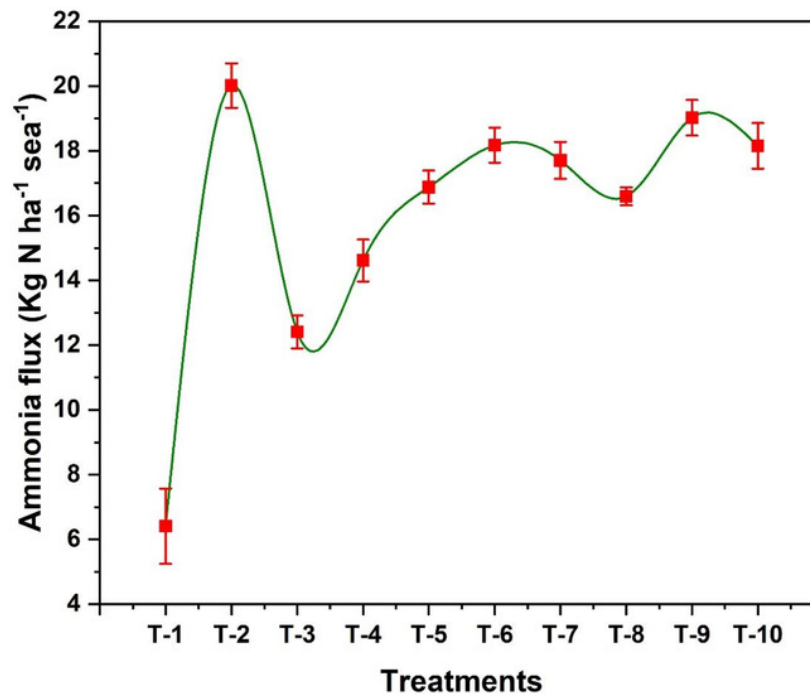
Figure 3

Figure.3 Effect of various organic and inorganic amendments on soil organic carbon (SOC) in the soil after the harvesting of wheat
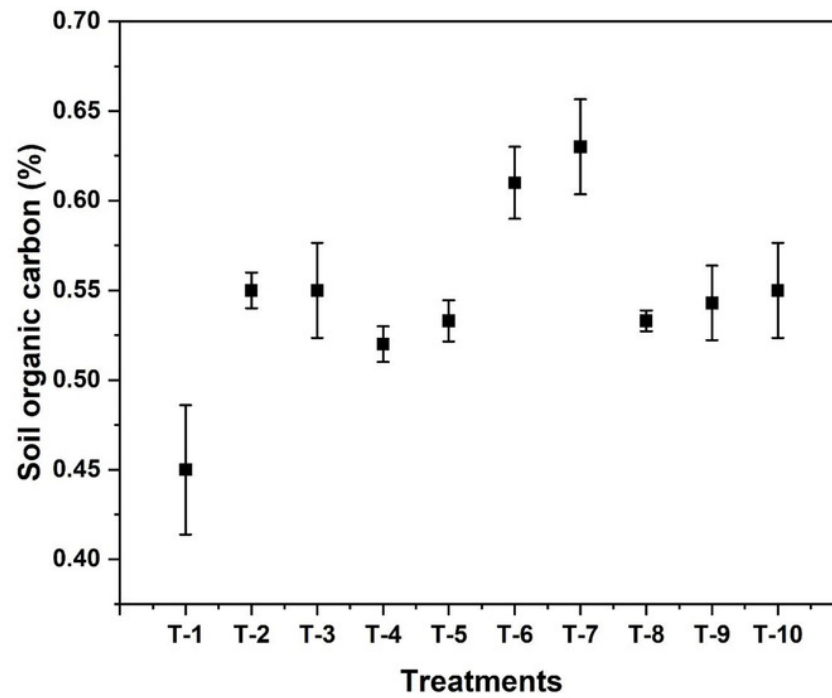
Figure 4

Figure 4. Effect of various organic and inorganic amendments on NH_4^+-N and NO_3-N concentration in soil
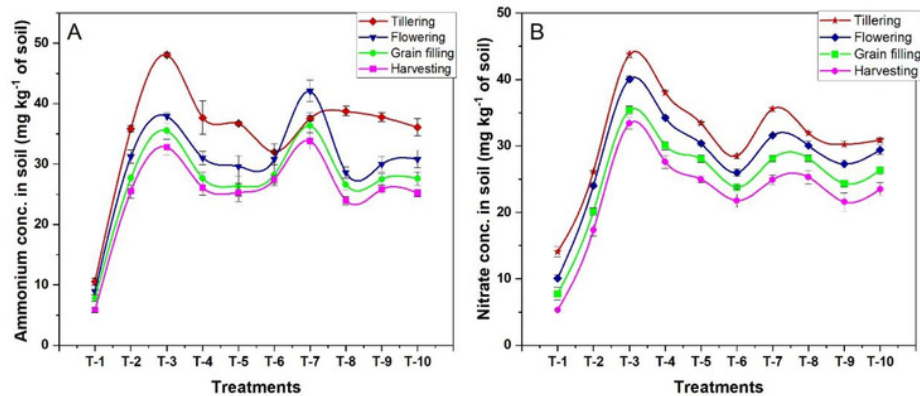
Figure 5

Figure 5. Effect of various organic and inorganic amendments on nitrous oxide flux and denitrification losses in soil
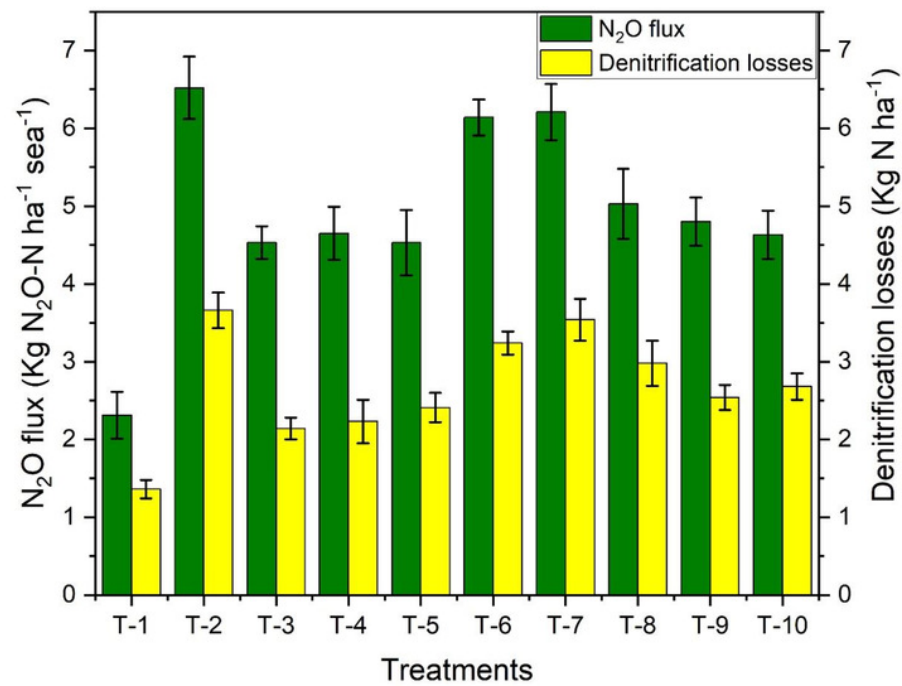
Figure 6

Figure 6. Effect of various organic and inorganic amendments on soil microbial biomass nitrogen and carbon (MBN and MBC) in soil
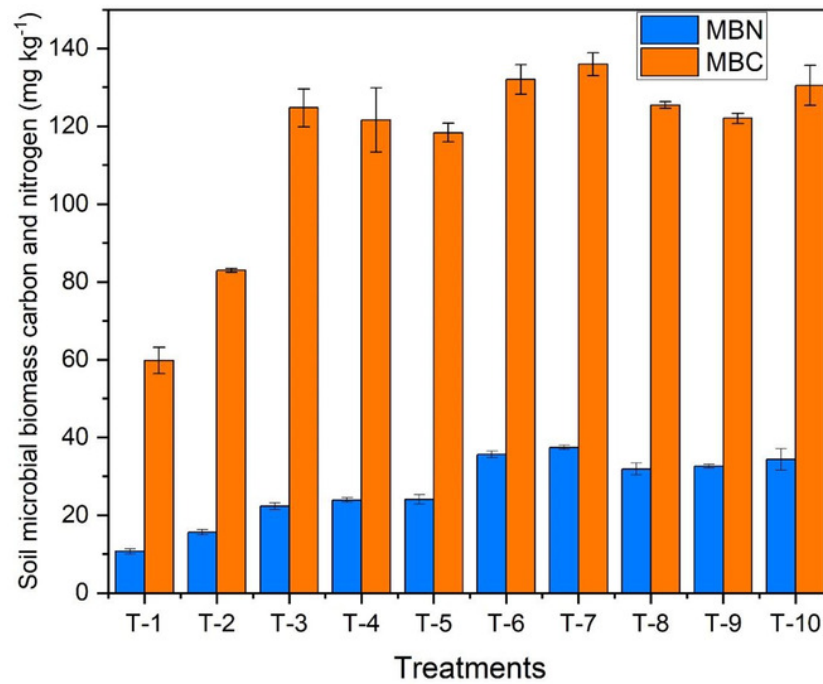
Figure 7

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