

1 **Response of co-inoculation of Phosphate Solubilizing Bacteria (PSB) and Arbuscular**
2 **Mycorrhizal (AM) fungi to microbial population of soil and NPK uptake by wheat**
3 **(*Triticum aestivum* L.) crop**

4 **ABSTRACT**

5 **Aim:** To study the response of co-inoculation of Phosphate Solubilizing Bacteria (PSB) and
6 Arbuscular Mycorrhizal (AM) fungi to microbial population of soil and nutrient uptake by wheat
7 crop .

8 **Study Design:** The used design was completely randomized design with three replications.

9 **Place of study:** The pot experiment was conducted during *Rabi* season of 2017 taking wheat (cv.
10 HD 2967) as test crop in experimental unit of the department of Soil Science and Agricultural
11 Chemistry, Bihar Agricultural College, Sabour, Bhagalpur (Bihar).

12 **Methodology:** The present study includes eight treatments with three replications. N P K uptake
13 by wheat crop were measured from each treatment and microbial population of soil were
14 determined from the rhizospheric soils collected from each treatment by using standard protocol.

15 **Results:** Maximum microbial population *viz.*, Bacteria, Actinomycetes, Fungi (39.00, 21.33,
16 24.66 cfu $\times 10^5$ g⁻¹ oven dry soil) were recorded under treatment T₄ (T₁+PSB @ 20 g kg⁻¹
17 seed+AM fungi @ 5.0g pot⁻¹) for bacteria and actinomycetes and T₃ (T₁+AM fungi@5.0 g pot⁻¹)
18 for fungi, at flowering stage and similar trend was followed at harvesting stage. The treatment T₄
19 significantly increased available nitrogen, phosphorus and potassium in soil as well as also
20 contributed to comparatively better plant growth and higher uptake of N, P and K by grain and
21 shoot. The maximum N, P and K content of wheat was also recorded under treatment T₄ which
22 was found to be most effective in modifying soil microbial population, microbial community
23 structure and grain yield of wheat crop.

24 **Conclusion:** Application of co-inoculation of Phosphorus solubilizing bacteria and arbuscular
25 mycorrhizal fungi enhance the microbial population and N P K uptake from soil by wheat crop.

26
27 **Keywords:** AM fungi, microbial population, phosphorus solubilizing bacteria, wheat

28
29 **Introduction**

30 Phosphorus is critical element for plant growth and their development, and is a
31 component of the nucleic acid structure of plants and biomembrane. Consequently, it is
32 important in cell division and tissue development. Phosphorus is also involved in the energy

33 metabolism of cells and is required for the biosynthesis of primary and secondary metabolites in
34 plants. Therefore, plants have evolved a range of strategies to increase phosphorus uptake and
35 mobility [9], the most common among which are phosphate solubilizing bacteria (PSB) and
36 Arbuscular mycorrhiza (AM) fungi symbiosis.

37 Most of the applied phosphorus in the soils is not taken up by the crop, but it is
38 retained in insoluble forms or fixed as mineral forms in the farms even as high as 90% or
39 more. Soil P can exist in various inorganic (Pi) and organic forms (Po). Specific determination of
40 Pi can be obtained by fractionation methods. Generally, plants take up P as the primary (H_2PO_4^-)
41 and secondary orthophosphate (HPO_4^-) ions. They are easily retained in most soils when added,
42 and in many cases this retention is so high that the element becomes largely unavailable to the
43 plants. The P retained by the soil is generally considered as fixed P, although a part of it can be
44 utilized. The rate of release of P from fixed form to replenish the immediately available soil
45 P is the most important factor in determining the P supplying capacity of the soil
46 because the quantity of P present in soil solution is not sufficient to meet the crop
47 requirements. The release of fixed soil P depends upon the nature of its fixation and the
48 extracting power of the crop or reagent used to determine availability of soil P. The P is
49 generally fixed as Fe-P and Al-P in acidic soils and Ca-P in alkaline soils.

50 P-solubilization ability of the microorganisms is considered to be one of the most
51 important traits associated with plant phosphorus (P) nutrition. Recently, phosphate solubilizing
52 microorganisms have attracted the attention of agriculturists as soil inoculums to improve the
53 plant growth and yield. Conversion of the insoluble forms of P to the form which is
54 available to plants (ortho-phosphate) is an important characteristics of phosphate- solubilizing
55 bacteria (PSB) and arbuscular mycorrhizal fungi (AMF). Bacteria such as PSB and AM fungi
56 are usually effective on phosphate solubility due to different mechanism such as
57 production and secretion of organic acids and by their co-inoculation they make
58 phosphorus available to plant for different metabolic functions [3]. Release of phosphorus by
59 PSB from insoluble and fixed or adsorbed forms is an important aspect regarding P
60 availability in soils. There are strong evidences that soil bacteria are capable of
61 transforming soil P to the forms available to plant. AM fungi are species of fungi that
62 intimately associate with plant roots forming asymbiotic relationship, with the plant

63 providing sugars for the fungi and the fungi providing nutrients such as phosphorus, to the
64 plants. Mycorrhizal fungi can absorb, accumulate and transport large quantities of phosphate
65 within their hyphae and release to plant cells in root tissue. Arbuscular mycorrhizal (AM)
66 fungus plant relationships are usually described as mutually beneficial, because fungi
67 supply mineral nutrients, especially phosphorus (P) to their host plants in return for
68 photosynthates. The contribution of AM fungi to P uptake by positively responsive plants
69 can be easily identified by comparison of P uptake in AM plants and nonmycorrhizal (NM)
70 plants. The beneficial effect of arbuscular mycorrhiza on plant growth is mainly attributed
71 to higher P uptake by plants [12].

72 This is the fact that phosphorus solubilizing bacteria and mycorrhizal fungi increase the
73 availability of phosphorus. There is a great scope to make more P availability in soil by adopting
74 new agronomic practices like use of co-inoculation of PSB and AMF with its appropriate dose,
75 proper combinations and inorganic fertilizer etc. Considering above mentioned facts a pot
76 experiment on response of Co-inoculation of Phosphorus solubilizing bacteria (PSB) and
77 Arbuscular Mycorrhizal (AM) Fungi on phosphorus availability under wheat rhizosphere has
78 been conducted.

79 **Methods and Material**

80 The present study was undertaken in pot to evaluate the response of co-inoculation of
81 Phosphate Solubilizing Bacteria (PSB) and Arbuscular Mycorrhizal (AM) fungi to soil microbial
82 population and availability of phosphorus under wheat rhizosphere during the *Rabi* season of
83 2017 with a promising var. HD 2967, at the experimental site of department of Soil Science and
84 Agricultural Chemistry, Bihar Agricultural University, Sabour, Bhagalpur, India.

85 The microbial inoculums viz., PSB- *Burkholderia cepecia* and AM fungi- *Glomus*
86 *mosseae* were procured from biofertilizer production unit, Bihar Agricultural University, Sabour,
87 Bhagalpur, and Bihar. The soil used for the pot experiment was Ustochrept clayey in texture,
88 having a pH of 7.78 and EC of 0.20 ds m⁻¹. The organic carbon content of the soil was 0.47%,
89 and the available nitrogen (N), phosphorus (P) and potassium (K) content was found 150.42,
90 12.51 and 192.66 kg ha⁻¹, respectively.

91 The following treatment structure was formulated for the study: T₁-RDF (120:60:40), T₂-
92 T₁+PSB @ 20 g kg⁻¹ of seed, T₃-T₁+AM fungi@5.0 g pot⁻¹, T₄-T₁+PSB@20 g kg⁻¹ seed+AM

93 fungi @ 5.0g pot⁻¹, T₅-75% RDF of Phosphorus +PSB@ 20 g kg⁻¹ seed, T₆-75% RDF of
94 Phosphorus +AM fungi @ 5.0g pot⁻¹, T₇-75% RDF of Phosphorus +PSB @ 20g kg⁻¹ of
95 seed+AM fungi@5.0 g pot⁻¹, and T₈-50% RDF of Phosphorus +PSB @ 20g/kg seed+AM fungi
96 @5.0 gPot⁻¹. Earthen pots of 15 cm height and 30 cm diameter were filled with 10 kg of soil.
97 The seed treatment with PSB@ 20 kg⁻¹ seed was done and AM fungi@ 5g Inoculum pot⁻¹ were
98 applied 2cm below the seed at the time of sowing. In each pot, 10 seeds of wheat (var. HD-2967)
99 were planted. Nitrogenous, phosphatic and potassic fertilizers were applied just before the
100 sowing according to the treatments. Using urea, single super phosphate and murate of potash as
101 source of nitrogen, phosphorus and potash, respectively and mixed in the soil uniformly by
102 working with spade. The irrigation were applied as and when required the crops. The plants were
103 thin to maintain eight plants in all pots.

104 Rhizosphere samples were drawn from the soil adhering to the roots. The 10 g of soil
105 samples were placed in an Erlenmeyer flask containing 90 ml of sterilized distilled water, and
106 shaken for 30 min. Ten-fold series dilutions were prepared, and appropriate dilutions were plated
107 in specific media. For the isolation of bacteria, fungi and actinomycetes, the Plate Count Agar,
108 Czapek-Dox Agar [19] and Kenknight and Munaier's Medium, respectively were used. The
109 numbers of colony forming cells were determined in each plot by serial dilution pour plate
110 method [18]. Phosphorus concentration in straw and grain were determined by employing the
111 vanadomolybdate yellow color method given by [6] and the distribution of P in soil was
112 determined by using a modified version of the [5]. Analysis of variance (ANOVA) was
113 performed as described by [2] to determine the effects of various treatments. Critical difference
114 (CD) at 5% level of probability and P values was used to examine differences among treatment
115 means.

116 **Result and Discussion**

117 **Effect on microbial population**

118 **Bacterial population**

119 It is evident from presented data that the microbial population resulted highest in the
120 flowering stage of wheat plant growth. This might be due to accumulation of various root
121 exudates and which in turn, established a strong and well defined root-microbe interaction [16].
122 The inoculation with treatment T₄ (T₁+PSB @20 g kg⁻¹ seed + AM fungi @ 5.0g pot⁻¹) having

123 significantly more bacterial population, when compared with applied treatments. It is clearly
124 shown that all applied treatments have given maximum bacterial count at flowering stage when
125 compared with harvesting stage. The application of treatment T₄ (T₁+PSB@20 g kg⁻¹ seed + AM
126 fungi @ 5.0g pot⁻¹) also produced significantly higher bacterial count by 22.23%, 4.28%, 9.41%,
127 than application of T₁ (RDF 120:60:40), T₂ (T₁ + PSB@20g kg⁻¹ seed), T₃ (T₁ + AM fungi@5.0g
128 pot⁻¹) respectively. The similar trends were observed at harvesting stage. At harvesting stage
129 application of treatment T₆ (75% RDF of P + AM fungi @ 5.0g pot⁻¹) and treatment T₇ (75%
130 RDF of P + PSB @ 20g kg⁻¹ seed + AM fungi @5.0g pot⁻¹) given significantly higher
131 colonization over all the applied treatments. Similar trend followed at harvesting stage. It might
132 be due to the AM fungi are probably the most abundant fungi in agricultural soil accounting for
133 somewhere between 5 and 50% of biomass of soil microbes live on carbohydrates obtained from
134 the root cells. They alter root exudation considerably [8] and are therefore expected to influence
135 rhizosphere populations as well [4]. Numerous studies have shown conclusively that AM is
136 having synergistic interaction with other beneficial soil microorganism such as N fixers and P
137 solubilizers. AM fungi affect the composition of bacterial communities either directly by
138 changing host plant physiology or indirectly by changing the pattern of root exudation. The
139 number of both rhizospheric bacteria and actinomycetes enhanced when plant formed
140 mycorrhizae, along with the inoculation of PSB [15]. There may be two pathways for AM fungi
141 to change microbe community structure, the first one is that the AM fungal hyphae secretion
142 directly impacts microbe community structures; the another one is that both AM fungi in
143 roots and on the roots alter plant physiological and biochemical processes, then directly or
144 indirectly change the plant root secretion, thus alter those structures [20].

145 **Effect on actinomycetes count**

146 Same trend of enhancement of actinomycetes count has been resulted under the
147 experimentation as compared as obtained in case of bacteria. The growth pattern was resulted
148 synonymous to bacterial counterpart. The highest individual treatment effect was observed under
149 the treatment T₄ (T₁ + PSB@20 g kg⁻¹ seed + AM fungi @ 5.0g pot⁻¹) in flowering stage,
150 produced maximum population of actinomycetes (21.33 cfu×10⁶ g⁻¹ soil). This treatment also
151 given significantly more actinomycetes population in all the applied treatments except treatment
152 T₂ (T₁+PSB @ 20g/kg of seed). Similar trends were follow at harvesting stage. The similar
153 results were obtained by [11] who conducted a field experiment and found that the maximum

154 bacterial population ($71.66 \text{ cfu} \times 10^5 \text{ g}^{-1}$ soil and $40.00 \text{ cfu} \times 10^5 \text{ g}^{-1}$ soil), fungi population
155 ($27.33 \text{ cfu} \times 10^4 \text{ g}^{-1}$ soil and $22.66 \text{ cfu} \times 10^4 \text{ g}^{-1}$ soil) and actinomycetes population ($57.66 \text{ cfu} \times$
156 10^5 g^{-1} soil and $46.33 \text{ cfu} \times 10^5 \text{ g}^{-1}$ soil) are observed in the treatment T₅ {(75 % N (FYM) basal
157 + 25 % N (V/C) at 25 DAT + *Azospirillum* @ 5 kg ha⁻¹ + PSB @ 5 kg ha⁻¹ + KSB @ 5 kg ha⁻¹)}
158 at both the panicle and harvesting stage in scented rice.

159 **Effect on fungi count**

160 It is depicted from the data that the fungal count resulted highest in the flowering stage of
161 growth as compared to harvesting stage. The application of treatment T₃ (T₁ + AM fungi @ 5.0 g
162 pot⁻¹) given maximum population of fungi ($24.66 \text{ cfu} \times 10^3 \text{ g}^{-1}$ soil). This treatment also given
163 significantly higher fungal population by 17.02%, 40.53%, 16.21 %, 44.59%, 12.16%, 16.21%,
164 over application of treatment T₁ (RDF (120:60:40), T₂ (T₁+PSB@ 20g kg⁻¹ seed), T₄ (T₁+PSB @
165 20 g kg⁻¹ seed + AM fungi @ 5.0g pot⁻¹), T₅ (75% RDF of P + PSB @ 20g kg⁻¹ seed), T₆ (75%
166 RDF of P + AM fungi @ 5.0g pot⁻¹) and T₇ (75% RDF of P + PSB @ 20g kg⁻¹ seed + AM
167 fungi@5.0 g pot⁻¹) respectively. Similar trend observed at harvesting stage. At harvesting stage
168 the maximum number of fungal population ($15.667 \text{ cfu} \times 10^3 \text{ g}^{-1}$ soil) was recorded with the
169 application of treatment T₃ (T₁ + AM fungi @ 5.0 g pot⁻¹). It might be due accumulation of
170 various root exudates and which in turn, established a strong and well defined root-microbe
171 interaction [10] also as compared to bacteria and fungi. The similar results were found by the
172 [16].

173 **.Response of PSB and AMF species to availability of nutrient in soil after harvest**

174 **Available Nitrogen (kg ha⁻¹)**

175 The maximum available nitrogen ($210.24 \text{ kg ha}^{-1}$) was recorded under the application of
176 treatment T₄ (T₁+PSB @ 20 g kg⁻¹ seed +AM fungi @ 5.0g Pot⁻¹). It has been clearly observed
177 that the available nitrogen in soil was significantly higher than all the applied treatments. It
178 might be due to the production of more shoot biomass and root biomass by the application
179 of given treatments and might be due to the structural changes in the microbial community.
180 These changes in the microbial community may alter the nutrients dynamics in the rhizosphere.
181 The similar results were observed by the [1].

182 **Available phosphorus (kg ha⁻¹)**

183 The data regarding available phosphorus in soil revealed that the maximum phosphorus
184 (19.25 kg ha^{-1}) was found by the application of treatment T₄ (T₁+PSB @ 20 g kg⁻¹ seed + AM

185 fungi @ 5.0g Pot⁻¹). The application of treatment T₄ given significantly higher available
186 phosphorus by 31.68% , 15.37%, 19.16%, 10.38% and 5.66% when compared with the
187 treatment T₁{ (RDF (120:60:40))}, T₂ (T₁+PSB @ 20g kg⁻¹ of seed), T₃(T₁+AM fungi @ 5.0 g
188 Pot⁻¹) ,T₅ (75% RDF of P +PSB @ 20g kg⁻¹ seed), T₆ (75% RDF of P +AM fungi @ 5.0g Pot⁻¹),
189 and numerically the least value of available phosphorus was found under the treatment T₁ (RDF
190 (120:60:40)). Recorded data shows that the inoculation with T₇ (75% RDF of P +PSB @ 20
191 g kg⁻¹ of seed + AM fungi @ 5.0 g Pot⁻¹) having also more significantly availability of
192 phosphorus by 28.72%, 11.70%, 15.66%, 6.50% and 22.76% over the treatments T₁ , T₂ , T₃ ,
193 T₅ and T₈ respectively. It may be due to the *Glomus mosseae* had pronounced effect for
194 phosphorus acquisition in soil inoculated with PSB have a great result. [13] who conducted
195 an experiment on *Coriander sativum* L. to study the effect of arbuscular mycorrhizal fungus
196 *Glomus mosseae* and phosphorus application on plant growth rate, essential oil content and
197 composition of coriander, and found that the mycorrhizal inoculation significantly increased
198 growth responses and P and N plant nutrients in shoot and root tissue, also after inoculation of
199 arbuscular mycorrhizal fungi in to coriander plant is a feasible alternative to increase growth
200 , nutrition, essential oil production and reduce the use of P fertilizers required to obtain economic
201 production of coriander under phosphorus deficient soil condition.

202 **5.3.3. Available potassium (kg ha⁻¹)**

203 The maximum available potassium (210.07 kg ha⁻¹) in soil was recorded by the
204 application of T₄ (T₁+PSB @ 20 g kg⁻¹ seed + AM fungi @ 5.0 g Pot⁻¹). This treatment also
205 gave significantly higher amount of available potassium in soil by 13.66%, 11.68%, 12.81%,
206 12.25% ,12.13%, 4.72% over the treatments T₁{(RDF (120:60:40))}, T₂ (T₁ + PSB @ 20 g kg⁻¹
207 seed), T₅ (75% RDF of P +PSB @ 20 g kg⁻¹ seed), T₆ (75% RDF of P +AM fungi @ 5.0 g Pot⁻¹
208), T₇ (75% RDF of P +PSB @ 20 g kg⁻¹ of seed + AM fungi @ 5.0 g Pot⁻¹) and T₈ (50% RDF of
209 P +PSB @ 20 g kg⁻¹ seed + AM fungi @ 5.0 g Pot⁻¹) while the treatment T₃ (T₁ + AM fungi @
210 5.0 g Pot⁻¹) given significantly zero value. It has been shown that in co-inoculated treatment of
211 PSB and AM fungi having more amount of available potassium present with respect to un-
212 inoculated condition like T₁, T₂ ,T₃ etc. Another treatment which is inoculated with T₈ given
213 significantly higher availability of potassium except T₃ and T₄ but another by 9.37%, 7.30%,
214 8.49% and 7.77% than T₁, T₂ , T₅ , T₇ respectively. The obtained results are in the agreement of
215 [1], who conducted an investigation to evaluate the response of selected species of mycorrhizae

216 for nutrient acquisition and phosphorus uptake by maize in an alluvial soils of Bihar and found
217 that value of available potassium has increased .

218 **Soil organic carbon (%)**

219 The data revealed with percent organic carbon in soil showed that the application of all
220 treatments were non-significant. The data regarding available organic carbon in a soil
221 maximum (0.54%) was recorded by the application of treatment T₂ (T₁+ PSB @ 20g/kg of
222 seed), T₃ (T₁+AM fungi @5.0 g Pot⁻¹), T₄ (T₁+PSB @20 g kg⁻¹ seed +AM fungi @ 5.0 g Pot⁻¹),
223 T₅ (75% RDF of P +PSB @ 20 g kg⁻¹ seed), T₆ (75% RDF of P +AM fungi @ 5.0 g Pot⁻¹), T₇
224 (75% RDF of P +PSB @ 20 g kg⁻¹ of seed + AM fungi@5.0 g Pot⁻¹) and T₈ (50% RDF of P +
225 PSB @ 20 g kg⁻¹ seed + AM fungi @5.0 g Pot⁻¹). It might be due to the AM colonization
226 produced more root biomass and plant biomass [1], who conducted an experiment Evaluation of
227 Arbuscular Mycorrhiza Fungi Species for Their Efficiency Towards Nutrient Acquisition in
228 Rhizospheric Soil of Maize and revealed that the organic carbon content exhibited significant
229 positive correlation with content. This positive correlation with organic carbon indicated that
230 cationic micronutrients formed complexes with organic matter and consequentially remained in
231 the forms, easily available to the plants.

232 **Effect of microbial inoculants on N P K content and uptake by wheat**

233 The data regarding N P K content and uptake in shoot and grain of wheat clearly
234 depicted that the application of treatment T₄ (T₁+PSB @ 20 g kg⁻¹ seed + AM fungi @ 5.0 g
235 Pot⁻¹) given maximum N P K content and their uptake over all the applied treatments, which is
236 found to be significantly higher over the applied treatment T₁ {(RDF (120:60:40)}, T₂ (T₁ + PSB
237 @ 20 g kg⁻¹ seed), T₅ (75% RDF of P +PSB @ 20 g kg⁻¹ seed), T₆ (75% RDF of P +AM fungi
238 @ 5.0 g Pot⁻¹), T₇ (75% RDF of P +PSB @ 20 g kg⁻¹ seed + AM fungi @ 5.0 g Pot⁻¹) and T₈
239 (50% RDF of P + PSB @ 20 g kg⁻¹ seed + AM fungi @ 5.0 g Pot⁻¹).

240 The data pertaining N content (%) has been clearly observed that the nitrogen content
241 percentage in shoot was maximum (0.149%) with the treatment T₄ (T₁+PSB@20 g kg⁻¹ seed+AM
242 fungi @ 5.0g pot⁻¹), which is significantly zero over the applied treatment T₁ {(RDF
243 (120:60:40)}, T₂ (T₁+PSB@20g kg⁻¹ of seed), T₃ (T₁+AM fungi@5.0 g pot⁻¹), T₅, (75% RDF of
244 Phosphorus +PSB @ 20g kg⁻¹ seed), T₆ (75% RDF OF Phosphorus +AM fungi @ 5.0g pot⁻¹) and
245 T₇ (75% RDF of Phosphorus +PSB @ 20g kg⁻¹ of seed+AM fungi@5.0 g pot⁻¹) The lowest
246 nitrogen content (0.140%) was found under the treatment T₁. While the treatment T₃ given

247 significantly higher value by 2.75%,2.06%,2.7% over the applied treatment T₁,T₂ and T₅
248 respectively while T₄ and T₈ are at par values. In the same way treatment T₈(50% RDF of
249 Phosphorus +PSB @ 20g kg⁻¹ seed+AM fungi @5.0 g pot⁻¹) have given significantly higher
250 value by 5.40%,4.05%,2.02%,4.72%,2.70%,over the applied treatment T₁,T₂,T₃,T₅ and T₇ while
251 the treatment T₄ have at par values.

252 The data pertaining P content (%) has been clearly observed that the phosphorus content
253 percentage was maximum (0.245%) with the treatment T₄(T₁+PSB@20 g kg⁻¹ seed+AM fungi @
254 5.0g pot⁻¹),which is found to be significantly higher over the applied treatment T₁{(RDF
255 (120:60:40)},T₂(T₁+PSB@20g kg⁻¹ of seed) T₃(T₁+AM fungi@5.0 g pot⁻¹),T₅(75% RDF of
256 Phosphorus +PSB @ 20g kg⁻¹ seed),T₆(75% RDF OF Phosphorus +AM fungi @ 5.0g pot⁻¹
257),T₇(75% RDF of Phosphorus +PSB @ 20g kg⁻¹ of seed+AM fungi@5.0 g pot⁻¹) and T₈(50%
258 RDF of Phosphorus +PSB @ 20g kg⁻¹ seed+AM fungi @5.0 g pot⁻¹) by
259 22.85%,1.22%,2.04%,6.93%,4.48%,19.18% respectively.While the treatment T₂ have given
260 significantly higher value by 21.90%,5.71%, 17.95% over the applied treatment T₁,T₅ and T₈
261 respectively,while the values of treatment T₂,T₃,T₇ are at par.

262 The data pertaining k content (%) has been clearly observed that the potassium content
263 percentage was maximum (1.16%) with the treatment T₄(T₁+PSB@20 g kg⁻¹ seed+AM fungi @
264 5.0g pot⁻¹),which is found to be significantly higher over the applied treatment T₁{(RDF
265 (120:60:40)}, ,T₂(T₁+PSB@20g kg⁻¹ of seed),T₅(75% RDF of Phosphorus +PSB @ 20g kg⁻¹
266 seed),T₆(75% RDF OF Phosphorus +AM fungi @ 5.0g pot⁻¹),T₇(75% RDF of Phosphorus
267 +PSB @ 20g kg⁻¹ of seed+AM fungi@5.0 g pot⁻¹) and T₈(50% RDF of Phosphorus +PSB @
268 20g kg⁻¹ seed+AMfungi@5.0 g pot⁻¹) by 13.79%, 9.48%, 11.20%,10.34%, 9.48%, 7.75%
269 respectively while the value of T₄ and T₇ are at par .The treatment T₃ have given significantly
270 higher value than 13.04%,1.904% over the applied treatments T₁ and T₂,while the values of T₃
271 and T₄ are at par. The data pertaining nitrogen uptake (g pot⁻¹), has been clearly observed that
272 the nitrogen uptake in shoot was maximum (0.0460%) with the treatment T₄(T₁+PSB@20 g kg
273 ⁻¹ seed+AM fungi @ 5.0g pot⁻¹) of table in which the nitrogen uptake was maximum which is
274 significantly higher than applied treatment T₁{(RDF (120:60:40)},T₂(T₁+PSB@20g kg⁻¹ of seed
275),T₃(T₁+AM fungi@5.0 g pot⁻¹),T₅(75% RDF of Phosphorus +PSB @ 20g kg⁻¹ seed),T₆(75%
276 RDF Of Phosphorus +AM fungi @ 5.0g pot⁻¹) T₇(75% RDF of Phosphorus +PSB @ 20g kg⁻¹ of
277 seed+AM fungi@5.0 g pot⁻¹) and T₈(50% RDF of Phosphorus +PSB @ 20g kg⁻¹ seed+AM

278 fungi @5.0 g pot⁻¹) by 21.73%, 10.86%, 17.39%, 13.47%, 13.26%, 17.39% and 14.78%
279 respectively. 6.57%, 6.44%, 17.10%, 13.55% respectively. While the treatment T₃ have given
280 significantly higher value 5.26% over the applied treatment T₁ while the value T₂, T₅ and T₈ are at
281 par.

282 Also from the data pertaining P uptake (g pot⁻¹) clearly observed that the phosphorus uptake
283 in shoot was maximum (0.0760%) with the treatment T₄(T₁+PSB@20 g kg⁻¹ seed+AM fungi @
284 5.0g pot⁻¹) of table in which the phosphorus uptake was maximum which is significantly
285 higher than applied treatment T₁{(RDF (120:60:40))}, T₂(T₁+PSB@20g kg⁻¹ of seed), T₃(T₁+AM
286 fungi@5.0 g pot⁻¹), T₅(75% RDF of Phosphorus +PSB @ 20g kg⁻¹ seed), T₆(75% RDF of
287 Phosphorus +AM fungi @ 5.0g pot⁻¹) by 35.52%, 6.57%, 6.44%, 17.10%, 13.55%,
288 17.63%, 29.73% respectively. The similar results reported by [15].

289 The data pertaining K uptake (g pot⁻¹) has been clearly observed that the potassium
290 uptake in shoot was maximum (0.362%) with the application of treatment T₄(T₁+PSB@20 g kg⁻¹
291 seed+AM fungi @ 5.0g pot⁻¹) of table in which the potassium uptake was maximum which is
292 significantly higher than applied treatment T₁{(RDF (120:60:40))}, T₂(T₁+PSB@20g kg⁻¹ of seed
293), T₃(T₁+AM fungi@5.0 g pot⁻¹), T₅(75% RDF of Phosphorus +PSB @ 20g kg⁻¹ seed), T₆(75%
294 RDF OF Phosphorous +AM fungi @ 5.0g/pot) by 27.07% and 14.91%, 6.077%, 21.27%,
295 19.33%, respectively. While the treatment T₃ have given significantly higher value by
296 22.35%, 9.41%, 16.17%, 14.11%, 17.94%, 15% over the applied treatment T₁, T₂, T₅, T₆, T₇ and
297 T₈ respectively with the value T₄ is at par. It clearly indicate that the potassium uptake was more
298 and more occur in inoculated species with respect to uninoculated treatment. It might be due to
299 the co-inoculation of PSB and AMF along with full dose of fertilizers, PSB secrete organic
300 acids of lower molecular weight and AM fungi make it more available to plants through its
301 hyphae and thus nutrient content and uptake concentration increases. Similar results were
302 obtained by [14], who conducted an experiment on Co-inoculation studies of vesicular
303 Arbuscular Mycorrhizal fungi (VAM) and Phosphate solubilizing bacteria (PSB) on nutrient
304 uptake of *Marsdenia volubilis* (T. Cooke) and found an excellent improvements in uptake of
305 nutrients like N, P, K, Ca, Mg, Fe, Mn and Zn concentrations in *Marsdenia volubilis* than single
306 application.

307 **Conclusion:**

308 The application of microbial inoculants in combination perform better than alone inoculation.
309 The results of the study revealed that the maximum microbial population *viz.*, Bacteria,
310 Actinomycetes, Fungi were found con-inoculation of PSB and AM fungi along with
311 recommended dose of phosphorus. The application of PSB@20 g kg⁻¹ seed+AM fungi @ 5.0g
312 pot⁻¹ along with 100% RDF significantly increased available nitrogen, phosphorus and potassium
313 in soil as well as also contributed to comparatively better plant growth and higher uptake of N, P
314 and K by grain and shoot. This treatment was found to be most effective in modifying soil
315 microbial population, microbial community structure and grain yield of wheat crop.

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372 **Table 1: Effect of Co-inoculation of PSB and AM fungi on microbial population under**
 373 **wheat rhizosphere**

Treatment	Bacterial count (CFU 10 ⁶ g ⁻¹ soil)		Actinomycetes count (CFU 10 ⁶ g ⁻¹ soil)		Fungi count (CFU 10 ⁴ g ⁻¹ soil)	
	Flowering stage	Harvesting Stage	Flowering stage	Harvesting Stage	Flowering stage	Harvesting Stage
T ₁	30.33	21.00	12.66	5.66	20.46	10.66
T ₂	37.33	28.66	20.67	8.67	14.66	8.67
T ₃	35.33	27.66	18.66	17.67	24.66	15.66
T ₄	39.00	29.33	21.33	11.67	20.67	9.67
T ₅	30.66	29.00	10.67	7.66	13.67	7.66
T ₆	31.66	31.00	9.67	8.66	21.66	13.67
T ₇	32.66	31.00	13.67	11.66	20.66	11.66
T ₈	29.33	20.00	8.66	6.67	22.66	11.67
C.D.(P=0.05)	1.671	1.51	1.671	1.008	1.008	1.008
C.V.	2.876	3.18	2.876	5.896	2.899	5.17

374 Note: T₁-RDF (120:60:40), T₂- T₁+PSB @ 20 g kg⁻¹ of seed, T₃-T₁+AM fungi@5.0 g pot⁻¹, T₄-
 375 T₁+PSB@20 g kg⁻¹ seed+AM fungi @ 5.0g pot⁻¹, T₅-75% RDF of P +PSB@ 20 g kg⁻¹ seed, T₆-
 376 75% RDF of P +AM fungi @ 5.0g pot⁻¹, T₇-75% RDF of P +PSB @ 20g kg⁻¹ of seed + AM
 377 fungi@5.0 g pot⁻¹, and T₈-50% RDF of P +PSB @ 20g kg⁻¹ seed + AM fungi @5.0 g pot⁻¹.

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380 **Table 2: Effect of co-inoculation of PSB and AM fungi on availability of N P K (kg ha⁻¹)**
 381 **and organic carbon in soil after wheat harvest.**

Treatment	Available N, P K (kg ha ⁻¹ soil)			O C (%)
	Nitrogen	Phosphorus	Potassium	
T ₁	188.19	13.15	181.37	0.53
T ₂	199.95	16.29	185.52	0.54
T ₃	198.36	15.56	208.18	0.54
T ₄	210.24	19.25	210.07	0.54
T ₅	197.24	17.25	183.14	0.54
T ₆	204.18	18.16	184.33	0.54
T ₇	204.52	18.45	184.57	0.54
T ₈	202.13	14.25	200.14	0.54
C.D.(P=0.05)	0.422	0.209	0.232	NS
C.V.	0.12	0.722	0.069	-

382 Note: T₁-RDF (120:60:40), T₂- T₁+PSB @ 20 g kg⁻¹ of seed, T₃-T₁+AM fungi@5.0 g pot⁻¹, T₄-
 383 T₁+PSB@20 g kg⁻¹ seed+AM fungi @ 5.0g pot⁻¹, T₅-75% RDF of P +PSB@ 20 g kg⁻¹ seed, T₆-
 384 75% RDF of P +AM fungi @ 5.0g pot⁻¹, T₇-75% RDF of P +PSB @ 20g kg⁻¹ of seed+AM
 385 fungi@5.0 g pot⁻¹, and T₈-50% RDF of P +PSB @ 20g kg⁻¹ seed+AM fungi @5.0 g pot⁻¹.

386 **Table 3: Effect of co-inoculation of PSB and AM fungi on N P K content (%) and N, P, K**
 387 **uptake (g pot⁻¹) by wheat plant**

Treatment	N, P, K content (%) in plant			N, P, K uptake(g pot ⁻¹) by plant		
	N	P	K	N	P	K
T ₁	0.140	0.189	1.00	0.0360	0.0490	0.264
T ₂	0.142	0.242	1.05	0.0410	0.0710	0.308
T ₃	0.145	0.240	1.15	0.0380	0.0711	0.340
T ₄	0.149	0.245	1.16	0.0460	0.0760	0.362
T ₅	0.141	0.228	1.03	0.0398	0.0630	0.285
T ₆	0.142	0.234	1.04	0.0399	0.0657	0.292
T ₇	0.144	0.235	1.05	0.0380	0.0626	0.279

T ₈	0.148	0.198	1.07	0.0392	0.0534	0.289
C.D.(P=0.05)	0.001	0.008	0.008	0.001	0.005	0.004
C.V.	0.402	0.936	0.426	0.395	0.456	0.389

388 Note: T₁-RDF (120:60:40), T₂- T₁+PSB @ 20 g kg⁻¹ of seed, T₃-T₁+AM fungi@5.0 g pot⁻¹, T₄-
389 T₁+PSB@20 g kg⁻¹ seed+AM fungi @ 5.0g pot⁻¹, T₅-75% RDF of P +PSB@ 20 g kg⁻¹ seed, T₆-
390 75% RDF of P +AM fungi @ 5.0g pot⁻¹, T₇-75% RDF of P +PSB @ 20g kg⁻¹ of seed+AM
391 fungi@5.0 g pot⁻¹, and T₈-50% RDF of P +PSB @ 20g kg⁻¹ seed+AM fungi @5.0 g pot⁻¹.

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UNDER PEER REVIEW