

Evaluating African rice (*Oryza sativa*) genotype yield increase through mycorrhizal fungi and rhizobium inoculation

ABSTRACT

Aims: The continuous use of expensive chemical fertilizers for improved upland rice yield has been associated with reduced grain yield, increased soil acidity and nutrient imbalance. Exploitation of beneficial microbial source as biofertilizers for use in bio-fixation and solubilization of fixed nutrients and plant growth promotion is seen as potential alternative.

Study design: A randomized complete block design laid out in a split-plot arrangement was conducted to evaluate whether growth and yield components of selected upland rice genotypes will be improved through rhizobium and mycorrhizal fungi inoculation.

Place and Duration of Study: The study was conducted at the Teaching and Research farm of the Federal university of technology, Akure Ondo state, Nigeria during the dry season of 2013.

Methodology: The experiment was a 3 x 5 factorial experiment with mycorrhizal fungi, rhizobium and without (control) and five upland rice genotypes (N-U-1, N-U-8, WAB 56-104, OFADA GR and MOROBEREKAN). The study was a split plot arrangement in Randomized Complete Block Design (RCBD), with AMF and rhizobium inoculation in the main plot, while genotypes were in the sub-plot, replicated three times. There were three main plots, each plot consist of 45 sub-plots with a size measurement of 2m x 1m and inter sub-plot spacing of 0.5m in between plots. A total of 50 plants were planted per sub plot and each sub-plot consists of 14 plants per row. Transplanted seedlings were planted with the soil slurry containing rhizobium and mycorrhizal fungi inoculum into planting holes in the field at two seedlings per stand, according to their respective plot at a spacing of 25cm x 25cm. Yield component data collected include; number of days to 90% maturity, number of days to 50% flowering; plant height at maturity, number of primary tillers per plot, number of grains per panicle, number of panicles, number of filled and unfilled spikelet, weight of 1000 filled grains and grain yield per plot.

Results: Result showed significant ($P < 0.05$) effect of rhizobium and mycorrhizal inoculation on rice yield and yield components. 61.4% increase in grain yield was observed in rhizobium inoculated genotypes when compared to 37.4% increase in mycorrhized genotypes and the un-inoculated control. WAB56–104 and N-U-8 had the best response amongst genotypes inoculated with rhizobium while genotypes WAB56-104 and MOROBEREKAN responded better amongst mycorrhized genotypes in relation to yield components.

Conclusion: The results from this study indicate that mycorrhizal fungi and rhizobium inoculation has beneficial effects on African rice yield components and could play an important role in improving African rice productivity. The adoption and use of these microbial organisms as an alternate source of nutrient coupled with reduced mineral fertilizer input by smallholder rice farmers could help mitigate serious economic and ecological problems associated with the use of mineral fertilizers.

Keywords: Mycorrhizal fungi; rhizobium; biofertilizer; upland rice; grain yield

1. INTRODUCTION

Rice (*Oryza sativa*) is a major staple food for millions of people in West Africa and the most in- demand staple amongst cereal crops in Nigeria's food basket [1]; [2]. Rice cultivation and production in Nigeria

18 has increased in recent times due to series of government initiatives, change in policies and increased
19 efforts towards self-sufficiency. However, there has been a considerable lag between production and
20 demand level with imports making up the shortfall. The low productivity of the rice production system in
21 Nigeria is due to a lot of factors such as socio-economic constraint, crop management system and lack or
22 no access to external inputs [3]. Rain fed upland rice production system is the dominant cultivation
23 system across several agro-ecological zones in Nigeria [2]. Several abiotic and biotic factors are
24 responsible for the low productivity of upland rice production system [4]. Studies have shown that nitrogen
25 deficiency, phosphorus fixation and drought are the leading constraints to upland rice production in
26 Nigeria [5]; [2]. Small holder farmers which form the bulk of rice growers in the country are unable to
27 realize the potentials of recently released improved high yielding African rice genotypes such as NERICA
28 which mine soil nutrients rapidly and have higher nutrient use efficiency than traditional genotypes.
29 Furthermore, smallholder farmers lack the financial capability to purchase chemical fertilizer to replenish
30 mined nutrient from the soil. Exploitation of microbial sources such as mycorrhizae fungi and rhizobium as
31 biofertilizers for rice growth promotion and increased yield have been previously tested due to their
32 excellent endophytic plant-microbe interactions [6];[7]. Arbuscular mycorrhizae fungi (AMF) are excellent
33 colonizer of plant roots. They help colonized plant in accessing water especially during dry spells and also
34 help in the uptake and solubilization of immobile soil nutrients while the plants supplies carbon as food
35 and energy source to the fungi in a symbiotic relationship [8]; [9]. Phosphorus (P) deficiency and fixation
36 can severely limits rice production; colonization of plant root with arbuscular mycorrhizal fungi (AMF) may
37 have an influencing effect on P uptake, plant growth and increased yield [10]. Rhizobium are largely
38 recognized for their role in nodule formation in leguminous crops through biological nitrogen fixation but
39 studies have also shown that they can be inoculated into non-leguminous crops such as rice, wheat and
40 maize for plant growth promotion and increase yield [11]; [6]; [7]. They are widely regarded as the most
41 efficient biofertilizer in relation to the quantity of nitrogen fixed. It has been estimated that 40-250 kg
42 N/ha/year could be fixed through the microbial activities of rhizobium [12]. Rhizobium is said to promote
43 plant growth through mobilization and fixation of nutrient, improving plant resistance to abiotic stress,
44 solubilization of nutrients in nutrient fixed soils, release of plant growth-hormones [7]; [10]; [13]; [14].
45 Therefore, coating African rice genotype seed or soaking seedlings in soil slurry with mycorrhizal fungi
46 and rhizobium inoculum before planting or transplanting could help in improving nitrogen and phosphorus
47 bioavailability and uptake in deficient soils which would help improve rice yields, increase economic return
48 to farmers and mitigate environmental pollution. However, studies conducted by [15], suggested that
49 response of rice genotypes to inoculation with beneficial organisms may differ due to specificity of plant-
50 bacterial and fungi associations, differences in root exudation and gas exchange efficiency. Therefore,
51 rice genotypes with the best response from inoculations with introduced or native beneficial organisms
52 should be selected for recommendation. With this in hindsight, the study was set up to evaluate the
53 performance of African rice genotypes inoculated with mycorrhizal fungi and rhizobium under field

54 conditions with a view to identify promising and best performing inoculated genotypes in terms of yield for
55 recommendation.

56

57 **2. MATERIAL AND METHODS**

58

59 **2.1. Description of Location and Experimental Site**

60 The study was conducted at the Teaching and Research farm of the Federal university of technology,
61 Akure Ondo state, Nigeria during the dry season of 2013. It is located between Latitude 5°08 10.5"E and
62 7°17' 59.2"N, and at elevation of 140 m above the mean sea level. It has an average annual rainfall
63 range of about 1613mm per annum and the annual mean temperature is 27°C. but it is equally important
64 to note that over the period of the two-year experiment, average annual rainfall fell to 1233mm and
65 temperature level increased to a mean average of 31°C due to fluctuations in climatic conditions. The Soil
66 at the experimental site was a Sandy clay loam classified under the soil order alfisol according to [16],
67 while the vegetation is tropical rain forest with an average relative humidity of between 56 and 59% during
68 the dry season.

69

70 **2.2. Planting of the seedling in the nursery**

71 The nursery stage was conducted at the screen house of the Department of Crop Soil and Pest
72 Management of the Federal University of Technology, Akure. Micro polythene pots of about 5cm in
73 diameter and 10cm in length were filled with topsoil and mixed with mycorrhizae (*Glomus intradices*) and
74 rhizobium strains (RACA 3/5/12) separately to form slurry at a weight of 50g per pot; this was done to
75 ensure maximum colonization of rice roots before transplanting. Rice seeds were sown at 2 seeds per
76 pot, the pots were made moist and maintained for about 14 days before germinated seedlings were
77 transplanted to the field.

78 **2.3. Management practices**

79 The area was plowed and cleared and pegged before transplanting, the experimental plots were laid out
80 and sectioned accordingly. Seedlings were transplanted with the soil slurry carefully to prevent root
81 damage and ensure optimal root colonization. Seedlings of each variety were planted in each designated
82 plot. There was no pre or post application of herbicides/pesticides and no basal or recommended fertilizer
83 application was added throughout the duration of the experiment. Weeding was done manually by hoe
84 and hand. All other recommended cultural management practices were followed to ensure good crop
85 stand and development. Harvesting was done manually when 80% of the grains in the upper portion of
86 the panicle are yellow and those at the base are hard dough stage. Harvested genotypes were threshed
87 separately.

88

89 **2.4. Pre-Planting Soil Analysis**

90 Soil samples were collected at depth of 0-15cm and bulked together prior to the determination of
91 chemical properties before planting to ascertain nutrient status of the soil, Soil pH was determined in

92 1:2.5 (soil: water) and KCl solution (1:1) using glass electrode pH meter. Soil organic matter was
93 determined according to [17] method. Total nitrogen in the soil was analysed using Kjeldahl method [18].
94 Available phosphorus was extracted using Bray-1 P followed by molybdenum blue colorimetry.
95 Exchangeable cation (K, Ca, Mg) were extracted with 1 N Ammonium Acetate K in the extract was
96 determined by flame photometry, Ca and Mg were determined by atomic absorption spectrometer (AAS).

97

98 **2.5. Experimental Design**

99 The experiment was a 3 x 5 factorial experiment with Arbuscular mycorrhizae, Rhizobium and without
100 (control) and five upland rice genotypes (N-U-1, N-U-8, WAB 56-104, OFADA GR and MOROBEREKAN).
101 The study was a split plot arrangement in Randomized Complete Block Design (RCBD), with AMF and
102 rhizobium inoculation in the main plot, while genotypes were in the sub-plot, replicated three times. There
103 were three main plots, each plot consist of 45 sub-plots with a size measurement of 2m x 1m and inter
104 sub-plot spacing of 0.5m in between plots. A total of 50 plants were planted per sub plot and each sub-
105 plot consists of 14 plants per row. Transplanted seedlings were planted with the soil slurry into planting
106 holes in the field at two seedlings per stand, according to their respective plot at a spacing of 25cm x
107 25cm³.

108 **2.6. Source and Application of Planting Materials**

109 The different rice seeds (genotypes) were acquired from Africa Rice Centre, International Institute of
110 Tropical Agriculture, Ibadan (IITA). The genotypes are the recently released improved high yielding
111 genotypes commonly grown by farmers in the ecological zone. Cultured Arbuscular mycorrhizae fungi
112 (*Glomus intaradices*) inoculum with soil as carrier and cultured Rhizobium strains (RACA 3/5/12) were
113 obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

114

115 **2.7. Culture Media and Growth Condition**

116 The pure cultures of rhizobium strain RACA 3/5/12 was obtained from the (microbial technology culture
117 department), International Institute of Tropical Agriculture, Ibadan Nigeria (IITA). Rhizobium sp, RACA
118 was isolated from root nodules of cowpea, the strain were characterized by biochemical and molecular
119 methods. Rhizobium sp, RACA was maintained on yeast extract manitol agar medium. The culture were
120 maintained by periodic transfer and stored in the refrigerator. Photomicrography was used to to examine
121 roots of rice genotypes inoculated with rhizobium roots for established colonization.

122 **2.8. Mycorrhizal Infection Determination**

123 Portions of rice roots were collected, using a clean knife from all the replicates, in plastic bottles for the
124 mycorrhizal infection determination before the grinding and storage in 50% ethanol. Mycorrhizal staining
125 in the roots was achieved by heating the root samples in 10% KOH, rinsing with distilled water and
126 soaking in 1% HCl for 10 minutes. Trypan blue solution was used to stain the roots. The roots were
127 soaked in the Trypan blue solution for 2 hours, and the stained roots were destained with 50% glycerol.
128 The grid-intersect method of [19] was used to evaluate the percentage of root infection.

129 **2.9. Data Collection**

130 Data collected were number of days to 90% maturity; this was counted from sowing to the time grains are
 131 ripe, Number of days to 50% flowering; this was counted from sowing to the time just before the booting
 132 of the panicle, Plant height at Maturity; this was done before harvest, height of 5plants per plot were
 133 measured by the use of a meter stick starting from the base of the plant to the tip of the panicle, Number
 134 of primary tillers per plot; this was counted using 5 plants at random, Number of grains per panicle; this
 135 was done by randomly selecting five stands per treatments and the grains were counted thereafter,
 136 Number of panicles; this was done by randomly selecting five stands per treatments, Number of filled and
 137 unfilled spikelet; the number of filled and unfilled spikelet per panicle was counted using five sample
 138 panicles taken at random, Weight of 1,000 filled grains (g); One thousand seeds were selected at random
 139 after oven drying at about 14% moisture content and weighed using a triple beam balance, Grain yield
 140 per plot (kg); this was taken by winnowing the grains after drying to approximately 14% moisture content.
 141 Only the filled grains per treatment were weighed, this was taken by converting the grain yield per plot
 142 into hectare using the formula ($[weight\ in\ grams/m^2]*10$) [20].

143

144 **2.10. Statistical Analysis**

145 The data collected were statistically analyzed, all data were checked prior to statistical analysis for the
 146 violation of ANOVA assumption, and means were separated using Duncan multiple range test. Genstat
 147 statistical package was used for the analysis.

148

149 **3. RESULTS**

150

151 **3.1. Pre-plant soil physico-chemical properties**

152 The pre-plant soil physico-chemical properties of the site are shown in Table 1. Analysis carried out on
 153 soil samples taken from the site prior to planting revealed that the soil contains 60.4%, 26%, and 13.6%
 154 sand, silt and clay respectively and falls into the textural class of sandy clay loam. The soil organic carbon
 155 and total nitrogen values were 1.90% and 0.28g/kg respectively which are below critical limit. The soil
 156 was acidic with a pH of 5.03 and has a cation exchange capacity (CEC) of 6.68; potassium level of
 157 2.04cmolk⁻¹; phosphorus level of 26.58mg/kg⁻¹; magnesium level of 2.13 cmolk⁻¹ and calcium level of
 158 2.51cmolk⁻¹. Analysis indicate the need for nitrogen and phosphorus fertilization as they are deficient in
 159 the soil and in an unavailable form for plant use, which justified the need for inoculation with biofertilizers
 160 which will help to increase nutrient availability and uptake for enhanced rice yield.

161

162 **Table 1. Physico-chemical properties of experimental soil before planting**

Soil properties	Values
Sand (%)	60.4
Clay (%)	26
Silt (%)	13.6

Textural class	Sandy clay loam
Nitrogen (g/kg)	0.28
Organic Carbon (%)	1.90
Organic Matter (%)	3.26
Calcium (cmol/kg)	2.51
Magnesium (cmol/kg)	2.13
Potassium (cmol/kg)	2.04
Phosphorus (mg/kg)	26.58
pH	5.03
CEC	6.68

163 *mean values are presented in the table (n = 4)

164

165

166 **Table 2.** Arbuscular mycorrhizae infection in roots of rice plants

167

Treatments	% Colonization
Mycorrhizae	86.5a
Rhizobium	41.3b
Control	30.9c

168 Means with the same letter in same column are not significantly different from one another using Duncan

169 Multiple Range Test (DMRT) ($P = 0.05$)

170

171

172

173 **3.2. Effect of arbuscular mycorrhizal inoculation rice root colonization**

174 The data in (Table 2) reveals maximum root colonization in rice genotype treatment inoculated with
 175 introduced mycorrhizal fungi (*Glomus intaradices*) (86.5%). It was also observed that rhizobium
 176 inoculated treatments also recorded a (41.3%) root colonization by native arbuscular mycorrhizae fungi
 177 while the un-inoculated treatments recorded the lowest root colonization (30.9%) by native mycorrhizal
 178 fungi.

179

180 **3.3. Effects of mycorrhizae and rhizobium inoculation on yield components of rice genotypes**

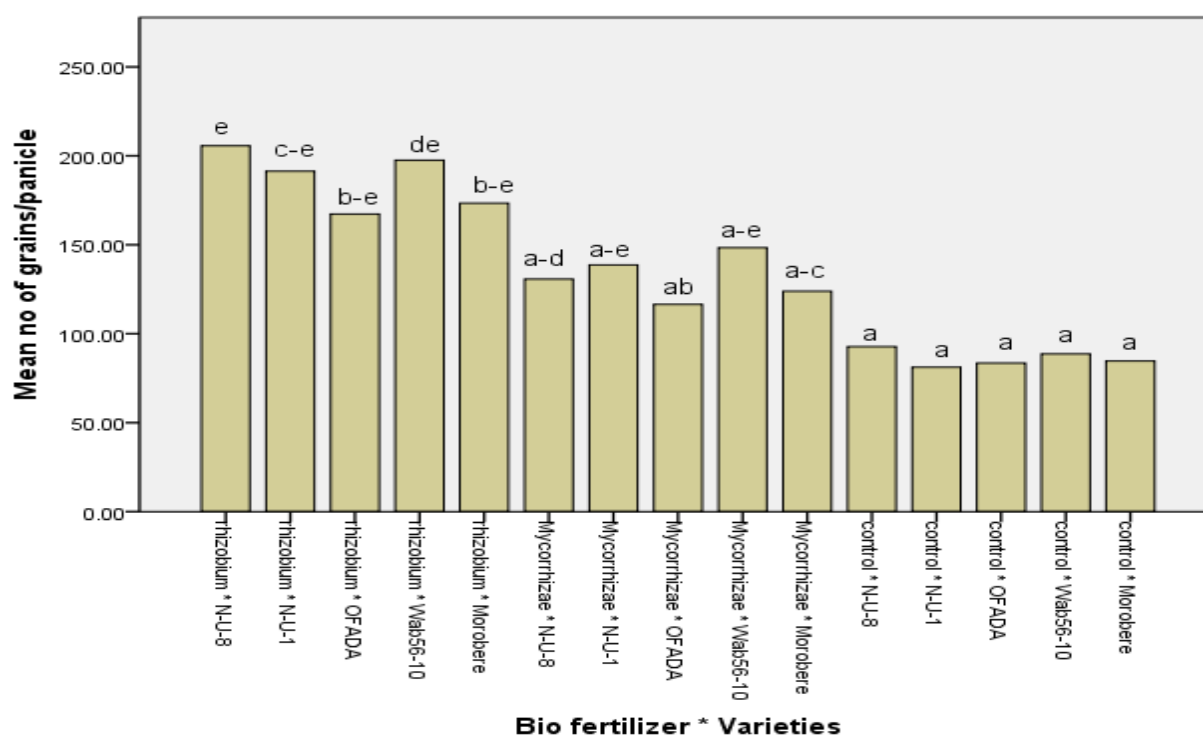
181 **3.3.1. Plant Height at Maturity**

182 The result presented in Table 3 indicate significant ($P < 0.05$) effect of rhizobium and mycorrhiza
 183 inoculation on plant height at maturity. Rice genotypes inoculated with rhizobium and mycorrhizae
 184 recorded higher plant heights (92.42cm and 86.18cm) respectively over the un-inoculated control
 185 (86.10cm). With respect to interactions between rice genotypes and biofertilizer treatments, no significant
 186 ($P < 0.05$) interaction was observed in plant height at maturity for both mycorrhized and rhizobium
 187 inoculated genotypes. However, rice genotype N-U-8 recorded the lowest plant height while Moroberekan
 188 a local rice genotype was the tallest and had the best response amongst all genotypes studied.

189

190 **3.3.2. Number of grains per panicle**

191 Significant ($P<0.05$) differences were observed with respect to number of grains per panicle. Rhizobium
 192 inoculated genotypes recorded the highest number of grains per panicle 187.05 and were significantly
 193 different from both mycorrhized genotypes (131.59) and the un-inoculated control (86.15). Mycorrhized
 194 genotypes were significantly different with higher number of grains per panicle recorded when compared
 195 with the un-inoculated control. Significant interaction ($P<0.05$) was observed between biofertilizers and
 196 rice genotypes (Fig 1), biofertilizer inoculated genotypes had better performance when compared with the
 197 un-inoculated control genotypes. Rice genotype (N-U-8) produced the highest number of grains per
 198 panicle and genotype (OFADA GR) the lowest number of grains per panicle amongst rhizobium
 199 inoculated genotypes. Rice genotype (WAB 56-104) produced the highest number of grains per panicle
 200 amongst mycorrhized genotypes.

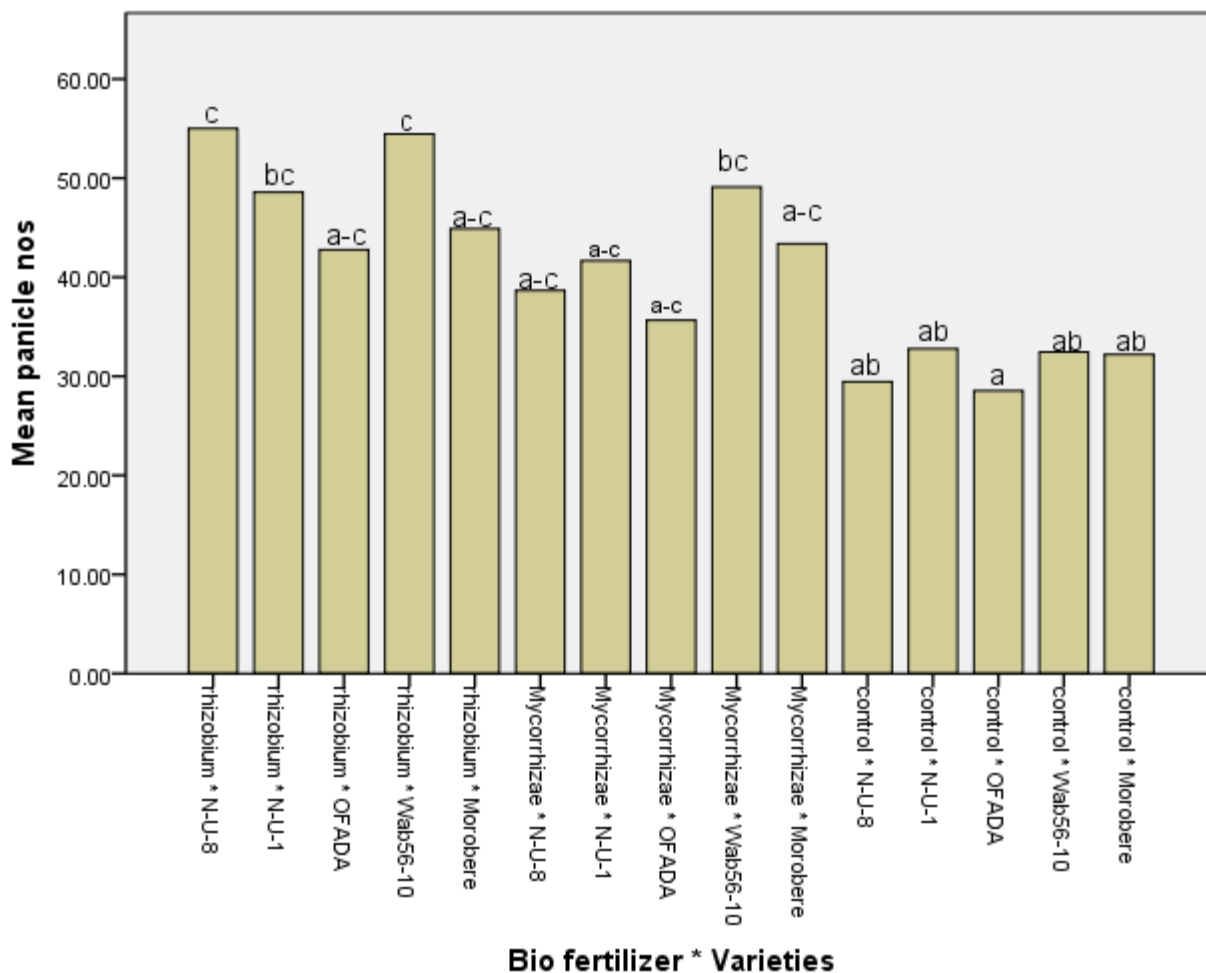


201 **Figure 1:** Interactive effect of mycorrhizae and rhizobium inoculation on rice genotypes and number of
 202 grains per panicle. Standard error ($P=0.05$)
 203
 204

205 **3.3.3. Number of panicle**

206 Significant ($P<0.05$) differences were observed in total number of panicles produced by rice genotypes
 207 (Table 3). Rhizobium inoculated genotypes recorded the highest panicle number (49.14) closely followed
 208 by mycorrhized genotypes (41.70) while the un-inoculated genotypes produced the lowest panicle
 209 number (31.08) Table 1. There was no significant ($P<0.05$) interaction observed between biofertilizer
 210 treatments and genotypes. However, rice genotype (N-U-8) and (WAB 56-104) produced the highest

211 panicle number and genotype (OFADA GR) produced the lowest panicle number in both treatments
 212 respectively.

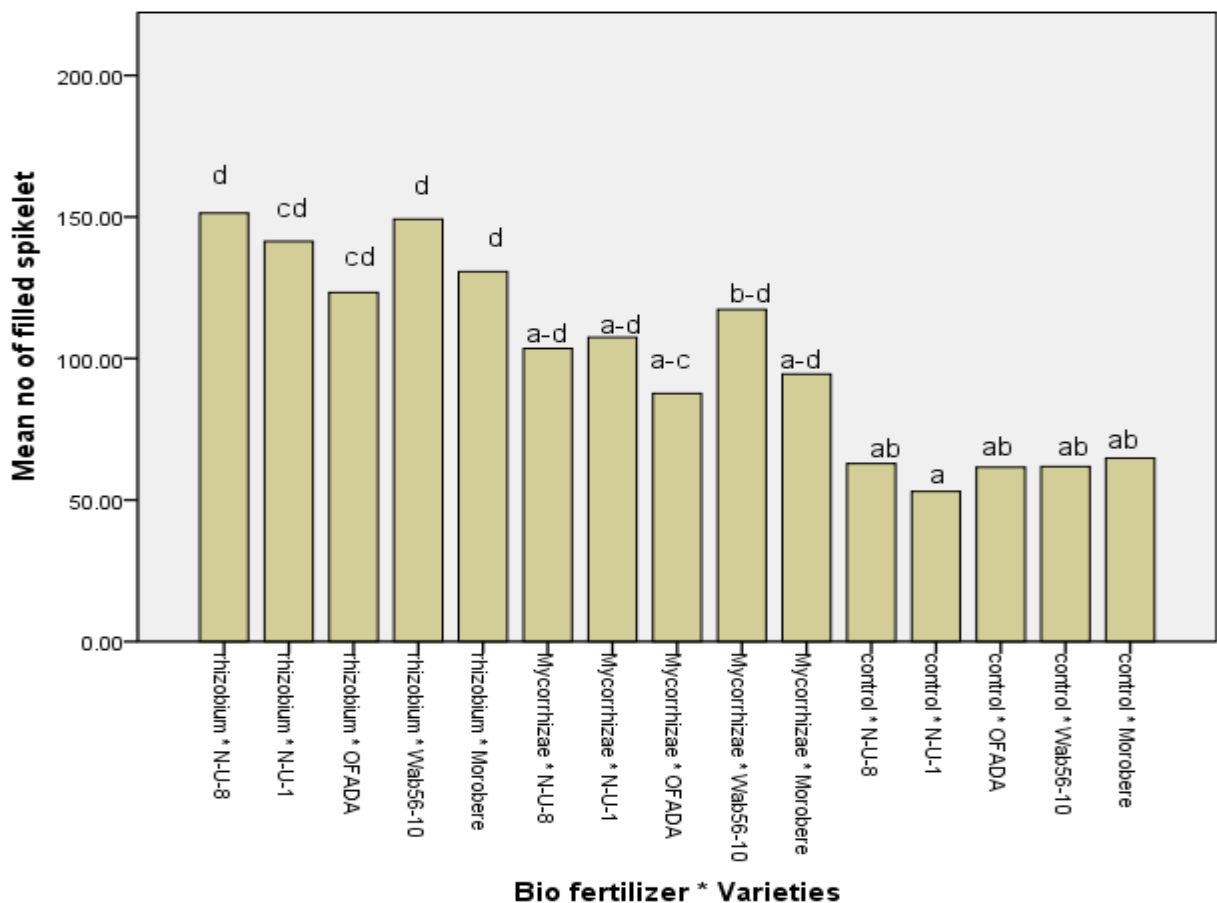


213
 214 **Figure 2:** Interactive effect of mycorrhizae and rhizobium inoculation on rice genotypes and number of
 215 panicles. Standard error (P=0.05)
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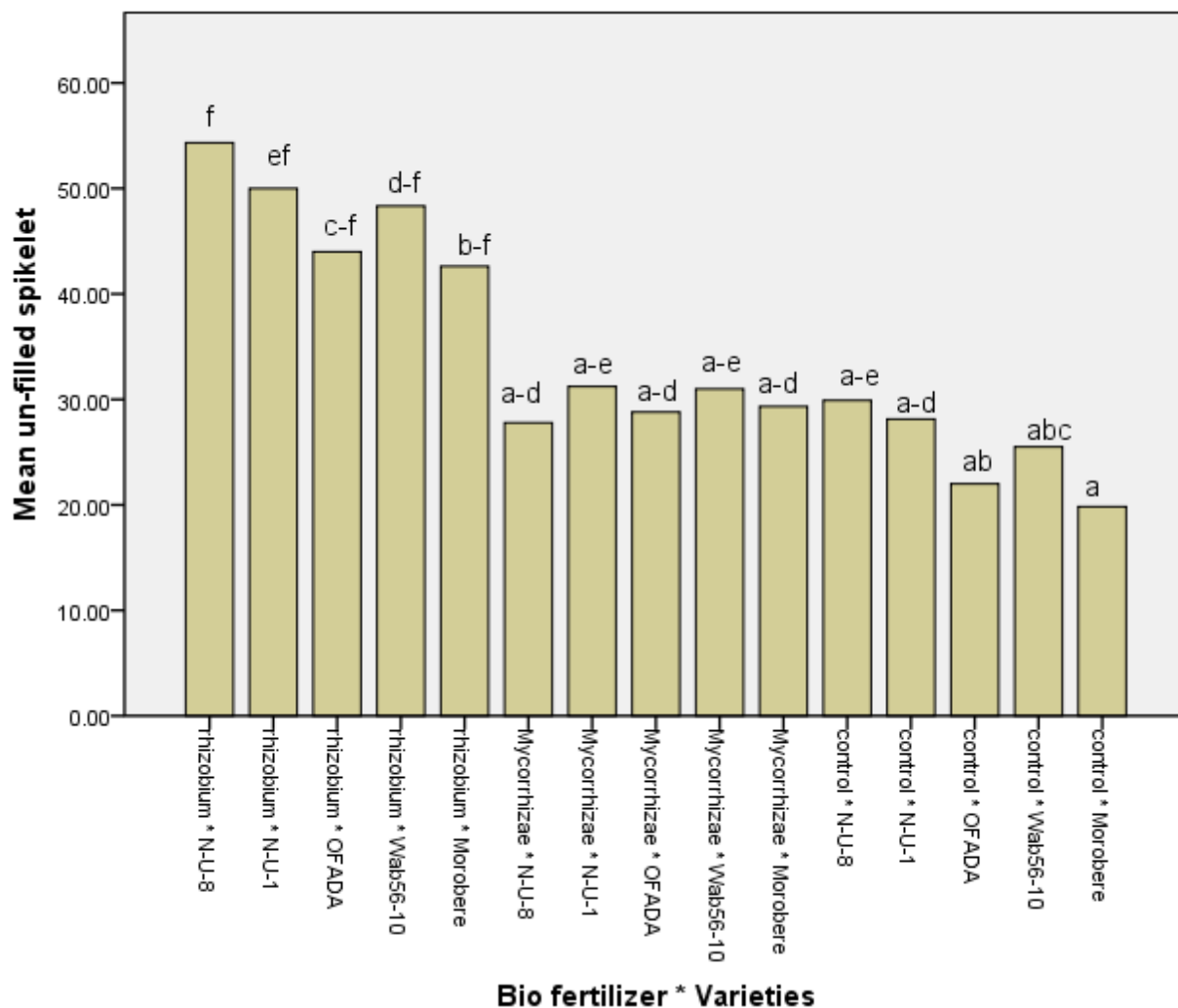
217
 218
 219 **3.3.4. Number of filled and unfilled spikelet**

220 Table 3, present the effect of rhizobium and mycorrhizae inoculation on number of filled spikelet and un-
 221 filled spikelet produced by inoculated rice genotypes. Significant ($P<0.05$) differences were observed
 222 amongst treatments, rhizobium inoculated genotypes had the highest filled spikelets (139.17) and un-
 223 filled spikelet (47.86), mycorrhized inoculated genotypes also recorded high filled spikelet number
 224 (102.10), it however produced lower un-filled spikelet number (29.63) than rhizobium inoculated
 225 genotypes. The Un-inoculated genotypes recorded the lowest filled spikelet number (60.81) and un-filled
 226 spikelet number (25.07). There was however no significant ($P<0.05$) interaction observed with respect to
 227 number of filled spikelet and un-filled spikelet in both mycorrhized and rhizobium inoculated genotypes

228 (Figs. 3 & 4). Rice genotype (N-U-8) produced the highest filled spikelet and genotype (OFADA GR)
 229 produced the highest unfilled spikelet amongst rhizobium inoculated genotypes. Amongst mycorrhized
 230 genotypes, no significant ($P < 0.05$) interaction was observed between biofertilizers and genotypes.
 231 However, rice genotype (WAB 56-104) recorded the highest number of filled spikelet, while genotype N-
 232 U-8 was recorded to have the highest number of unfilled spikelet.
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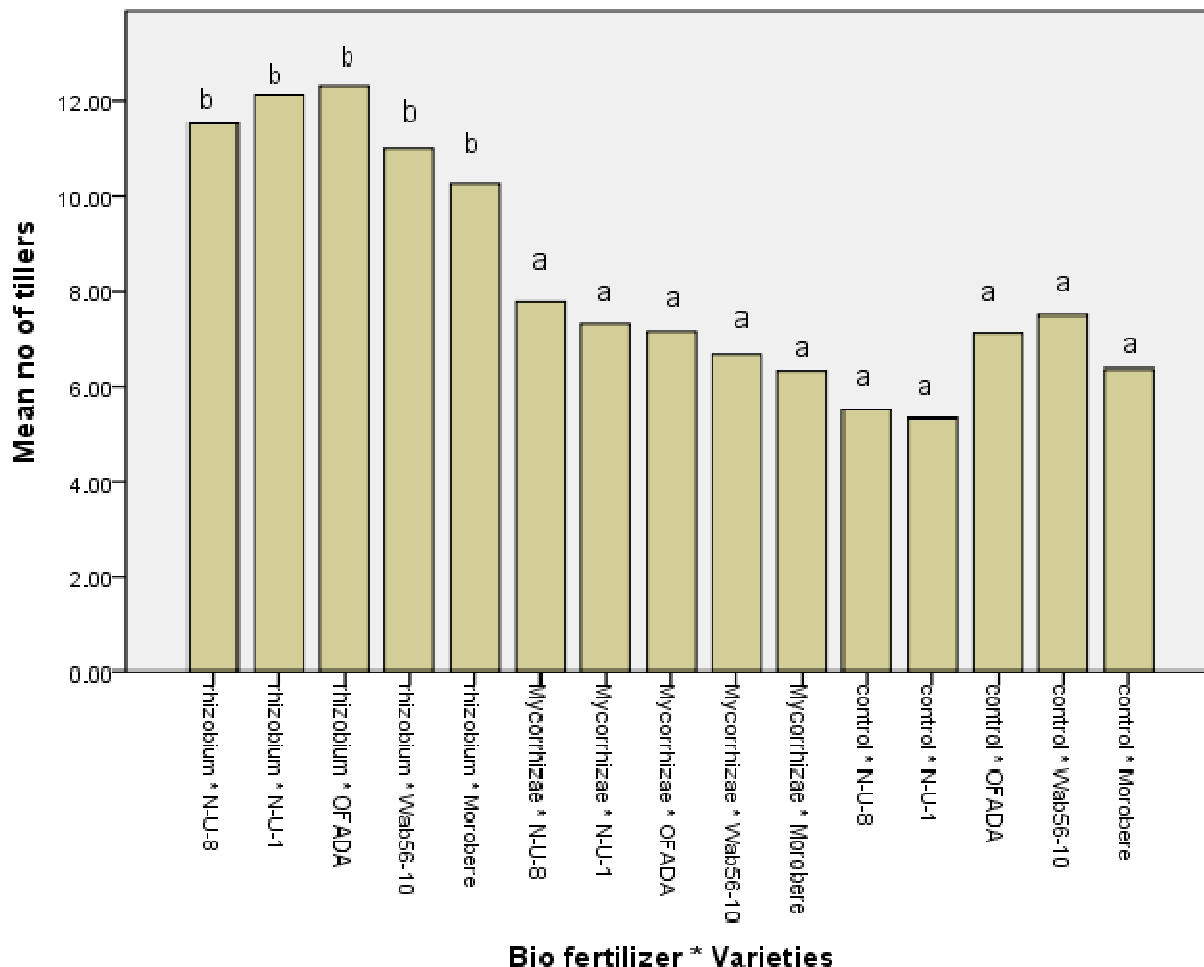
234
 235 **Figure 3:** Interactive effect of mycorrhizae and rhizobium inoculation on rice genotypes and number of
 236 filled spikelets. Standard error ($P=0.05$)
 237



238
 239 **Figure 4:** Interactive effect of mycorrhizae and rhizobium inoculation on rice genotypes and number of
 240 unfilled spikelet. Standard error ($P=0.05$)
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242 **3.3.5. Number of primary tillers**

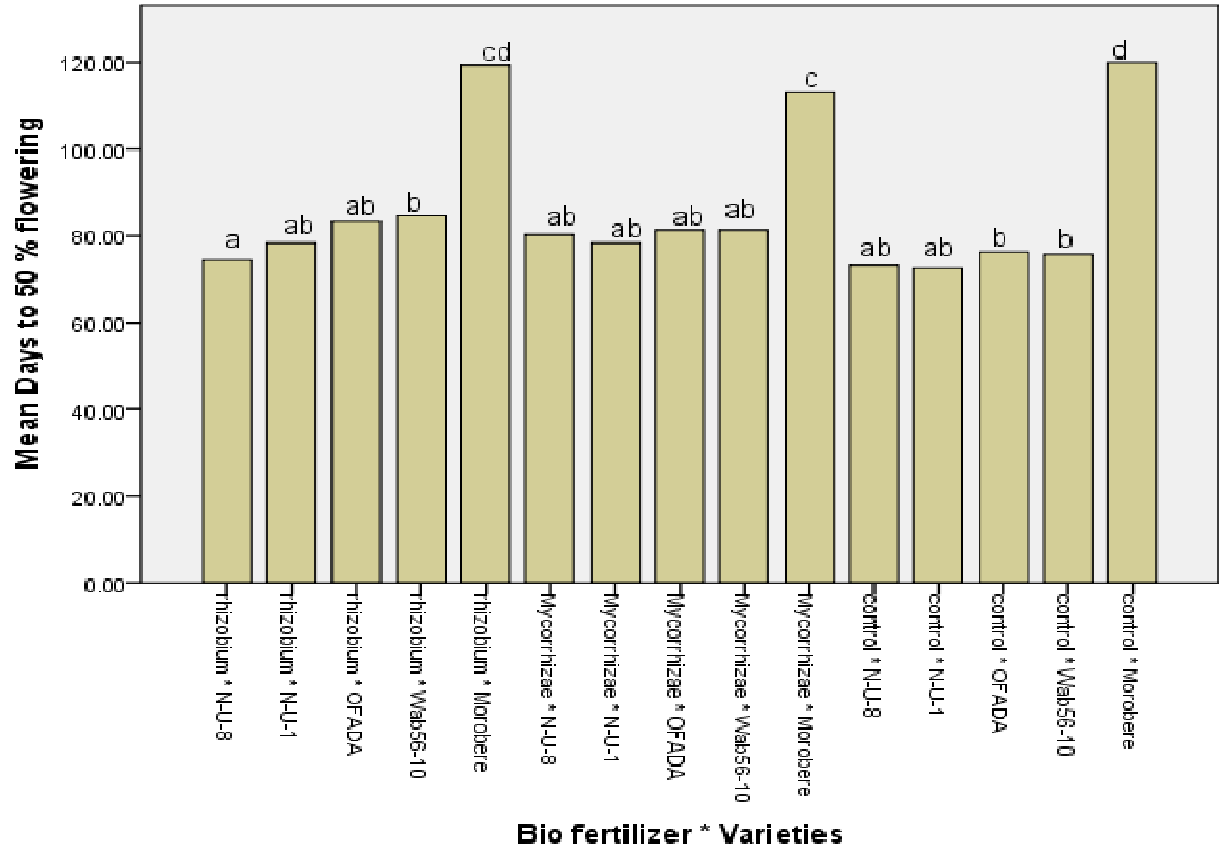
243 Significant ($P<0.05$) differences were observed in treatments with respect to number of primary tillers
 244 produced by rice genotypes (Table 3). Rhizobium inoculated genotypes produced more tillers (11.46)
 245 when compared with un-inoculated genotypes (6.37). Mycorrhized genotypes also produced higher tiller
 246 numbers (7.06) than the un-inoculated genotypes but was not significantly different. With respect to
 247 interactions between treatments and genotypes (Fig 5), OFADA GR produced the highest number of tiller
 248 amongst rhizobium inoculated genotypes; N-U-8 produced the highest number of tillers amongst
 249 mycorrhized genotypes while MOROBEREKAN recorded the lowest tiller numbers in both rhizobium
 250 inoculated and mycorrhized genotypes respectively.



251
 252 **Figure 5:** Interactive effect of mycorrhizae and rhizobium inoculation on rice genotypes and number of
 253 primary tillers. Standard error (P=0.05)
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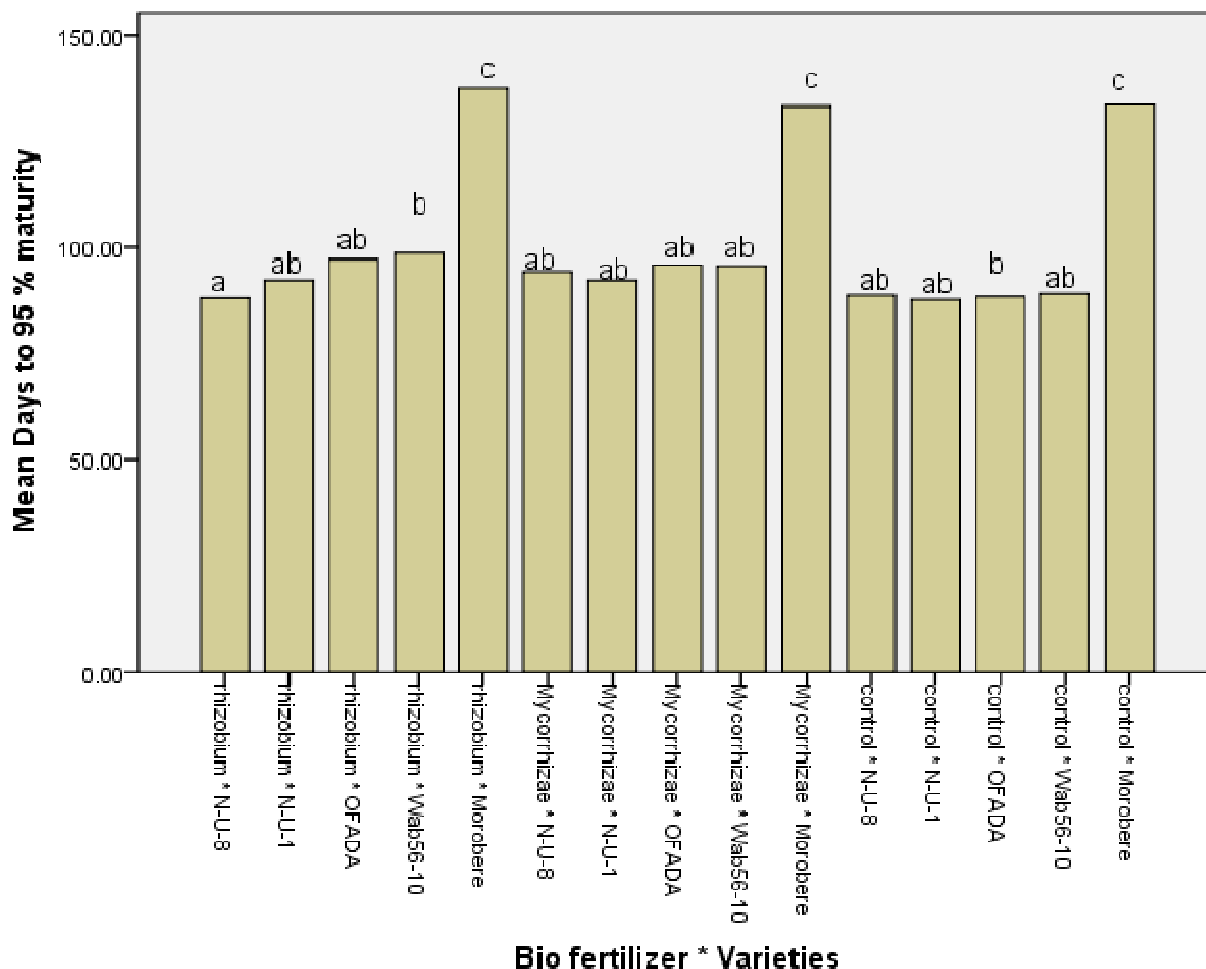
255 **3.3.6. Numbers of days to 50% flowering and 90% maturity**

256 There was no significant ($P < 0.05$) difference observed with respect to days-to-50% flowering and days-to-
 257 90% maturity amongst treatments (Table 3). However, results indicate that the un-inoculated treatment
 258 flowered and matured earlier (71.40 and 85.20 days respectively) than mycorrhizal treatment (86.87 and
 259 102.33 days) and rhizobium treatments (88 and 102.80 days respectively). Fig. 6 and 7 shows the
 260 significant interaction between biofertilizers and genotypes in reaching days to 50% flowering and days to
 261 95% maturity. Significant ($P < 0.05$) interaction was only recorded in days to 50% flowering for both
 262 rhizobium inoculated and mycorrhized genotypes. Rice genotype (N-U-8) flowered the earliest amongst
 263 rhizobium inoculated genotypes, while genotype (MOROBEREKAN) flowered late. With respect to days to
 264 90% maturity, genotype N-U-8 also matured the earliest and MOROBEREKAN matured late. In
 265 mycorrhized genotypes, significant ($P < 0.05$) interaction was observed with respect to days to flowering,
 266 N-U-1 flowered earlier while MOROBEREKAN flowered late.



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Figure 6: Interactive effect of mycorrhizae and rhizobium inoculation on rice genotypes and days to 50% flowering. Standard error (P=0.05)

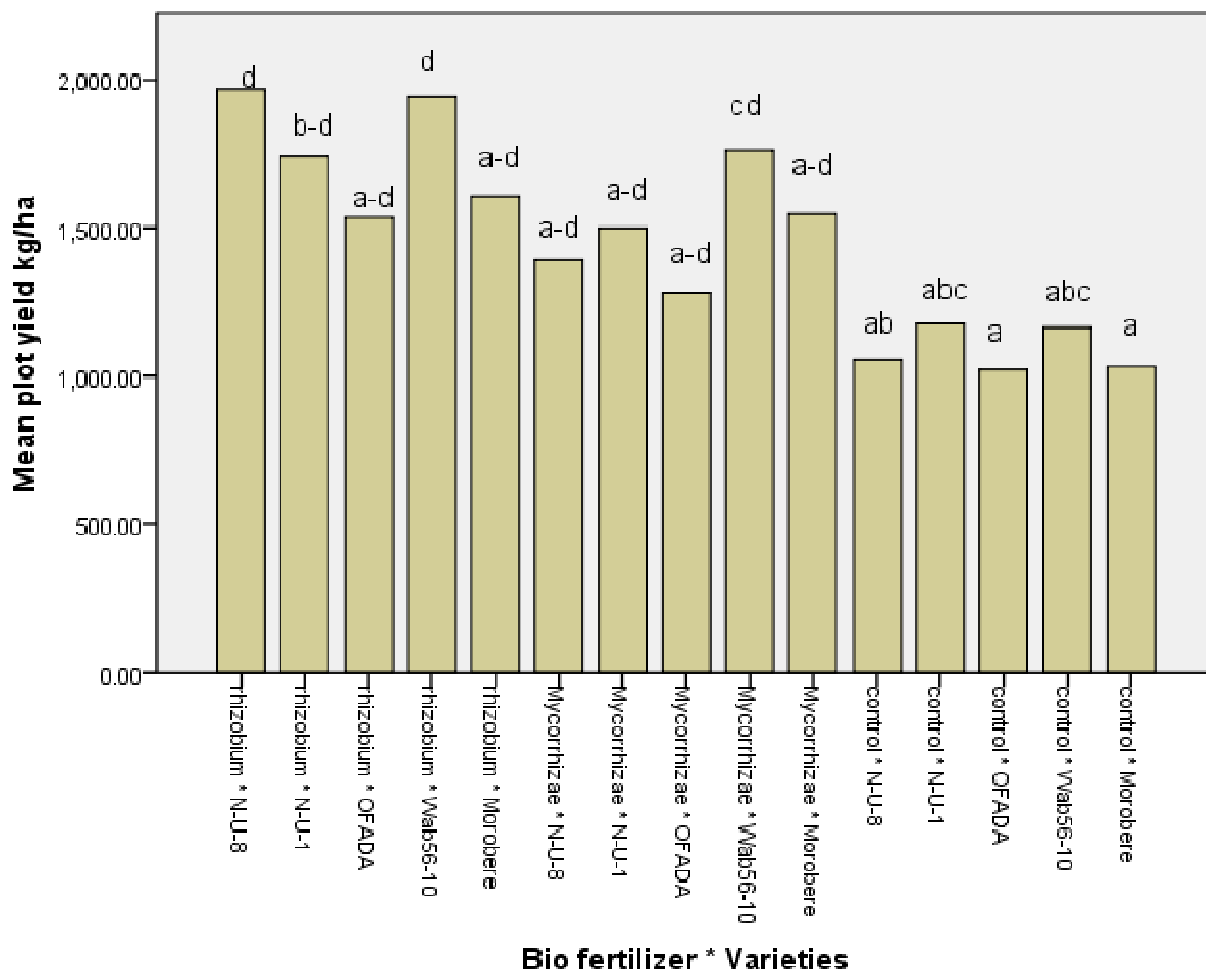


271 **Figure 7:** Interactive effect of mycorrhizae and rhizobium inoculation on rice genotypes and days to 95%
 272 maturity. Standard error ($P=0.05$)
 273
 274

275 **3.3.7. Grain yield and 1000 grain weight**

276 Table 3, shows the significant ($P<0.05$) effect of treatments on grain yield and weight of rice grain
 277 produced. Rhizobium inoculated treatments recorded higher grain yield than mycorrhizal treatment and
 278 the un-inoculated control treatment. Mycorrhizal treatments however had higher 1000 grain weight which
 279 wasn't significantly different from rhizobium treatment and the un-inoculated treatment. There was no
 280 significant ($P<0.05$) interaction observed in rhizobium inoculated genotypes with respect to grain yield
 281 (Fig 8). Rice genotype (N-U-8) produced the highest grain yield while genotype (OFADA GR) produced
 282 the lowest. With respect to 1000 grain weight, no significant ($P<0.05$) interaction was observed between
 283 treatment and genotypes however, genotype N-U-8 and N-U-1 produced the highest grain weight while
 284 genotype (MOROBEREKAN) produced the lowest grain weight. Amongst mycorrhized genotypes, no
 285 significant ($P<0.05$) interaction was observed with respect to grain yield. However, genotype (WAB 56-
 286 104) produced the highest grain yield. Significant ($P<0.05$) interaction was observed between

287 mycorrhizae and genotypes for 1000 grain weight. Rice genotype (N-U-1) recorded the highest 1000
 288 grain weight while genotype (WAB 56-104) weighed the lowest.



289
 290 **Figure 8:** Interactive effect of mycorrhizae and rhizobium inoculation on rice genotypes and grain yield.
 291 Standard error (P=0.05)
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296
297

Table 3. Effects of Mycorrhizae and Rhizobium inoculation on yield and yield components of rice

Treatments	Number of grains/panicle	Panicle Number	Number of filled spikelet	Number of un-filled spikelet	Plant height at maturity (cm)	Number of primary tillers	Number of days to 50% flowering	Number of days to 95% maturity	Grain yield (kg/ha)	Computed yield (t/ha)	1000 grain weight (g)
Control	86.15a	31.08a	60.81a	25.07a	88.10b	6.37a	71.40a	85.20a	1089.60a	2724a	27.1a
Rhizobium	187.05c	49.14c	139.17c	47.86b	92.42b	11.46b	88.00a	102.80a	1759.20c	4398c	29.6b
Mycorrhizae	131.59b	41.70b	102.10b	29.63a	86.18a	7.06a	86.87a	102.33a	1497.60b	3744b	30.1b

298 *Means with the same letter in same column are not significantly different from one another using Duncan Multiple Range Test (DMRT) (P = 0.05)

299 4. DISCUSSION

300
301 There has been scanty experimental evidence in Nigeria on the ability of Rhizobium which are normally
302 associated with leguminous crops and the ability of arbuscular mycorrhizal fungi (AMF) to colonize roots
303 of certain cereals e.g. rice, in nutrient deficient soils and promote their growth and yield. Increased
304 interest in nitrogen fixing bacteria associated with cereals such as rice, wheat and maize has been shown
305 in recent times to reduce the use of expensive mineral fertilizers in cereal production. One of the reasons
306 for the success recorded with nitrogen fixation independent of nodule formation in rice studies is the
307 observation that nitrogen status of rainfed and irrigated lowland soils under rice cultivation has increased
308 due to the activities of nitrogen fixing bacteria which survived under such condition evident with increased
309 growth and population of beneficial microbes [21]; [22]. Inoculation and improvement of cereals through
310 nitrogen fixing bacteria have been observed in many field studies [23]; [3]; [4]; [24]. Yield increase
311 observed in rice plants could be attributed to biological N fixation and also to production of plant growth
312 promoting hormones by the root colonizing organism. Increases in yield of wheat by inoculation with
313 *Azospirillum* strain Cd were consistent in field evaluations conducted in Israel and other semi-arid regions
314 [25]; [26]. It is however pertinent to note that cereal crop genotypes may portray significant differences in
315 their ability to associate with nitrogen fixing bacteria or mycorrhizal fungi. In two genotypes of sorghum,
316 inoculation with three strains of *Azospirillum* promoted an increase in dry plant weight and total N in grain
317 [27]. However, [27], also reported that rice plants inoculated with *A. lipoferum*, AI 121 and *A. brasilense*
318 did not influence rice growth or grain yield. A field study conducted by [28] in which rice plants were
319 inoculated with nitrogen fixing bacteria indicated that addition of low input of mineral N fertilizer increased
320 rice yield, nitrogen use efficiency and biological nitrogen fixation under flooded lowland conditions.
321 Results from this present study indicate that development and yield components of upland rice were
322 significantly ($P<0.05$) affected by inoculation with mycorrhizae and rhizobium with inoculated genotypes
323 recording higher statistical values over the un-inoculated control, with the rhizobium inoculated rice
324 recording a 61.4% increase in grain yield over the un-inoculated rice genotypes. The results established
325 the effectiveness of the introduced rhizobium strain for improving the development and yield of high
326 yielding NERICA genotypes and the other two indigenous genotypes used in the study. The increase in
327 growth, development and yield parameters in response to rhizobium inoculation endorsed the fact that
328 they have one or more growth and yield promoting mechanisms. The increase in studied characters might
329 be due to improvement in soil nutrient availability and nutrient uptake due to the secretion of auxins or
330 hormones plus N fixation by bacteria inoculation [29]; [32]. The results of this study are in agreement with
331 [11] who reported 16% increase in number of panicles and grains/panicle per plant of rice and suggested
332 that the improvement was due to increased availability of nutrients and phytohormones like indole acetic
333 acid and ethylene. The increase in 1000 grain weight observed with inoculations with rhizobium and
334 arbuscular mycorrhizae could be attributed to reduced spikelet number produced by inoculated genotypes
335 which consequently resulted in increased grain filling due to adequate amount of photosynthetic material
336 assimilated [30]; [31]. The result of this study also agrees with [33] who observed up to 23.63% increase

337 in developments of rice such as number of grains per panicle, filled spikelets, panicle lengths and tillering
338 over un-inoculated control and argued that indole acetic acid and gibberellins production could be the key
339 mechanism for that improvement. Maximum yield in inoculated plants may be attributed to the symbiotic
340 relationship of rhizobium (bacteria) with the roots of the plants, which fixed atmospheric nitrogen into the
341 roots of rice and thus the yield was increased. Early flowering and maturity observed in the un-inoculated
342 control than inoculated genotypes is suggested to be an induced phenotypic response to limiting abiotic
343 stress, such as moisture stress and high temperature. Mycorrhizae inoculated genotypes was observed
344 to have benefitted greatly through increased yield component and also a 37.4% increase in grain yield.
345 This positive influence on inoculated genotypes could be attributed to increased phosphorus, nitrogen
346 uptake, phytohormones such as cytokinins, essential micro-nutrients e.g Fe, Zn, Cu by rice plants which
347 lead to better development response and yield. The result was in agreement with [5], who reported that
348 inoculation of AMF resulted in comparatively better performance in growth, development and yield of
349 some selected drought tolerant upland rice genotypes investigated in the rainforest transitory zone of
350 Nigeria. However in the un-inoculated control, where the soil was phosphorus and nitrogen deficient and
351 no biofertilizer added the plants grew poorly and yield was low. The potential benefit of exploiting this
352 endophytic plant-bacterium association for cereal production also extends to decreased environmental
353 pollution and health risks originating from excessive use of mineral N fertilizers to achieve high grain yield
354 [6]. Finally the study has demonstrated that the single use of rhizobium and arbuscular mycorrhizae fungi
355 can enhance rice growth and yield through changes induced in growth physiology and root morphology of
356 rice genotypes. Further studies are required to test this study across differing agro-ecologies and use of
357 more genotypes and different strains of rhizobium and mycorrhizae for efficient selection and appropriate
358 recommendation.

359

360 **5. CONCLUSION**

361

362 This study reveals that inoculation with biofertilizers resulted in comparatively better performance in
363 relation to yield components of African rice genotypes inoculated than the un-inoculated. The yield of
364 genotypes N-U-8, N-U-1, WAB56-104, OFADA Gr and MOROBEREKAN were statistically similar
365 irrespective of the different biofertilizer treatment applied. However, single rhizobium inoculated
366 genotypes had slightly marginal better performance over mycorrhizal inoculated genotypes. In rhizobium
367 inoculated genotypes, WAB56–104 and N-U-8 had the best response, while in mycorrhizal inoculated
368 genotypes, WAB56-104 and MOROBEREKAN recorded better response with respect to yield. Further
369 investigation should be carried out to ascertain reported synergistic effect and performance of dual
370 inoculation of mycorrhizae and rhizobium on rice plants as only single inoculation of both bio fertilizers
371 was used in this study.

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375 **References**

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