

3 **Effect of Biofertilizer on growth and yield characteristics of**
4 ***Zea mays* L. in different ecological zones in Kenya**

11 **ABSTRACT**

In order to reduce the dependence on chemical fertilizers, alternative methods should be developed which will provide nutrients to plants. The increased cost of inorganic fertilizers, including their inability to condition the soil and their polluting effect on the environment, has directed attention towards other sources of soil fertilization to enhance maize production. Hence, this study was carried out to determine the effect of Effective Micro-organisms (biofertilizer) comprising *Pseudomonas spp*, *Saccharomyces spp*, *Bacillus subtilis* and *Lactobacillus spp*. on the growth and yield components of *Zea mays* L. The trial plots measured 4m×3m and the experimental design was randomized complete block design (RCBD) with 4 treatments namely; Biogrovit (biofertilizer) alone; conventional fertilizer alone, Biogrovit plus conventional fertilizer combined and the control. It was replicated three times. Biogrovit was soil drenched in plants at an interval of 14 days in crops established at two sites in Kirinyaga and Machakos County. Significant differences were observed in the leaf area where application of the biofertilizer had the largest (995 cm²) while the least was under the control (529 cm²). The grain yield was notably influenced by application of treatments at both sites, where the highest was recorded under the biofertilizer in Kirinyaga (8.6 t/ha) and Machakos (7.77 t/ha) which was not significantly different from that of the conventional fertilizers in Kirinyaga and Machakos at 7.55 t/ha and 6.87 t/ha respectively. The control had the lowest grain yield in both sites. The 1000-grain mass, ear length, cob weight, number of cobs per plant and the number of kernels per cob directly influenced the actual grain yield as they were higher for both biofertilizer and chemical treatments. The application of Biofertilizer and conventional fertilizer combined at full rates were antagonistic as most of the parameters tested had lower counts than when independently applied. Therefore, the findings of this study suggest that biofertilizers enhance the growth of maize and as such its use should be encouraged because it is eco-friendly.

13
14 *Keywords: Biofertilizer; Pseudomonas spp; Saccharomyces spp, Bacillus subtilis;*
15 *Lactobacillus spp.; Zea mays L.*

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18 **1. INTRODUCTION**

19 Maize is a valuable cereal that is highly cultivated in Nigeria because of its domestic and
20 industrial use. According to Tollenaar and Dwyer, [1], maize is the third most important
21 cereal crop after wheat and rice in the world based on area and production. The productivity
22 of maize is dependent on its nutrient requirement and management, particularly that of

23 nitrogen, phosphorus and potassium [2]. The increased cost of conventional fertilizers
24 including their inability to condition the soil and their polluting effect on the environment has
25 directed attention towards other sources of soil fertilization to enhance maize production.
26 Micro-organisms are involved in a range of processes that affect the transformation of soil
27 phosphorus and atmospheric nitrogen into usable forms for plant growth. Microbial
28 inoculants of bacteria, algae and fungi, either separately or in combination enhance the
29 availability of nutrients to plants by nitrogen fixation and solubilizing phosphorus for the
30 benefit of plants. Microorganisms are therefore critical in the conversion of atmospheric
31 nitrogen and transfer of phosphorus from accessible soil pools to plants in available forms.

32 Biofertilizers are preparations containing live or latent cells of efficient nitrogen fixing,
33 phosphate solubilizing algae, bacteria or fungi. The application of biofertilizers can be either
34 to the seed or soil to speed up microbial processes in the soil thereby augmenting the
35 availability of nutrients which can be assimilated by crop plants. Indeed, certain soil micro-
36 organisms have the inherent capacity to dissolve part of the bound phosphorus and make it
37 available to crops by secreting organic acids such as acetic acid, succinic acid, lactic acids,
38 etc. [3]. These attributes make the micro-organisms important as biofertilizers. The plant
39 promoting rhizobacteria can influence plant growth directly through the production of
40 phytohormones and indirectly through nitrogen fixation and production of biocontrol agents
41 against the soil-borne pathogens [4].

42 The use of microbial inoculants as biofertilizers has become a hope for most countries as far
43 as economic and environmental viewpoints are concerned. Biologically fixed nitrogen is such
44 a source that can supply an adequate amount of nitrogen to plants and other nutrients to
45 some extent [5]. It is a non-hazardous way of fertilization of the field. Moreover, biologically
46 fixed nitrogen consumes about 25% to 30% less energy than the chemical fertilizers. The
47 application of biofertilizers provides effective implementation of biological mechanisms of
48 plant nutrition, growth promotion and protection [6]. In order to reduce the dependence on
49 chemical fertilizers, an alternative method is to be developed which will provide nutrients to
50 plants. Through effective research and technology, Biogrovit combines useful micro-
51 organisms primarily; Lactic acid bacteria, photosynthetic bacteria, yeast, pseudomonas and
52 actinomycetes leading to a soil friendly, organic solution for improving soil fertility and
53 restoring pH balance in the soil. Each of these micro-organisms has an important role
54 complementing each other and are mutually beneficial. They collectively work towards the
55 betterment of the soil, environment and plants. However, there is limited information on the
56 advantages of biofertilizers prompting this study on maize in Kirinyaga and Machakos
57 Counties in Kenya.

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59 **2. MATERIAL AND METHODS**

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61 **2.1 Study sites**

62 There were three crops grown (Maize, French beans and Kale) in two locations of two
63 counties of Kenya namely Mwea in Kirinyaga County and Kitengela in Machakos County.
64 The maize variety used was DKC-9089 from Monsanto Seed Company.

65 **2.2 Experimental design and application of treatments**

66 The trial was laid out in a randomized complete block design (RCBD). The treatments
67 included; Biogrovit (biofertilizer) alone, conventional (chemical) fertilizer alone, Biogrovit plus
68 conventional fertilizer, and no application (control). The experimental plots measured 4m

69 long and 3m wide with a 0.5m pathway between plots and 1m pathway between blocks. The
70 treatments were replicated three times.
71 The land was ploughed and re-ploughed at a two-week interval then harrowed to a fine tilth
72 where the experimental units were demarcated. Furrows were opened at a spacing of 75cm
73 by 30cm for maize. Biogrovit was soil drench-applied near the plants at one week after
74 emergence and after every two weeks for five times to physiological maturity. A knapsack
75 sprayer at full pressure at a rate of 2 litres per acre was used after mixing with water in ratio
76 of 1 litre of Biogrovit in 100 litres of water. The conventional treatment had DAP fertilizer
77 applied at planting and top-dressed with CAN at the 5-leaf stage of the crop. All other
78 agronomic practices were carried out uniformly as recommended for the respective crops.

79 **2.4 Data collection and Statistical analysis**

80 Plant height was measured vertically based on the distance from the stem base to the
81 highest growing point of leaf segregation while the leaf number included standard counting
82 of leaves per plant including discoloured ones for cases of senescence. The leaf area was
83 measured using a leaf area meter Model. The cob weight and the number of rows per cob
84 were determined. To determine grain yield, biomass yield and harvest index, we removed
85 and cleaned all the seeds produced within 1m² central rows in the field. Then grain yield and
86 biomass yield were recorded on a dry weight basis. Yield was defined in terms of grams per
87 square meter and quintals per hectare. Replicated samples of clean seed (broken grain with
88 foreign material removed) were sampled randomly and 1000-grain were counted and
89 weighed.

90 The data collected was statistically analyzed by using the computer statistical program SAS
91 package. Analysis of variance technique was employed to test the overall significance of the
92 data, while the least significance difference (LSD) test at P= 0.05 was used to compare the
93 differences among treatment means [7].

94 **3. RESULTS AND DISCUSSION**

95 **3.1 Growth components**

96 In both sites, a conclusive trend observed among the test treatments showed differences,
97 with the absolute control having the lowest growth rate of maize in both sites. The leaf area,
98 number of leaves per plant and plant height significantly (P= 0.05) differed between the
99 treatments where the highest were under the biofertilizer and conventional treatments while
100 the lowest were under the control in both sites (Table 1 & 2). The tallest plants were
101 recorded under the biofertilizer treatments at 160cm and 158cm for Kirinyaga and Machakos
102 respectively while the shortest were under the control at 102.5cm and 93.8cm for Kirinyaga
103 and Machakos respectively. Also, the leaf area was highest under the biofertilizer and
104 conventional fertilizer treatments in both sites with the control exhibiting the lowest in both
105 sites. The average number of leaves per plant was significantly different between the
106 treatments where the highest in Kirinyaga (16) was recorded under the biofertilizer and
107 conventional fertilizer and the highest number of leaves per plant in Machakos was under
108 the biofertilizer treatment (18). There were no significant differences between the treatments
109 on the stand count in both sites.

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111 Table 1. Influence of test treatments on growth parameters of maize at Kirinyaga

Treatment	Leaf Area	Leaf	Height (cm)-80	Stand
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	(cm ²)	Number	DAP	Count
Biogrovit	995a	16.3a	160.0a	26a
Biogrovit+Conventional	643c	15.3ab	132.3b	24a
Control	529d	14.4b	102.5c	17a
Full Conventional	752b	15.5ab	155.0a	20a
SE	74.6	0.369	2.826	2.612
CV%	12.9	9	6.4	15.1
F pr.	<.001	<.001	<.001	0.173

112 Means followed by the same letter in each column are not significantly different at P≤0.05.
113 DAP-Days after planting

114 Table 2. Influence of test treatments on growth parameters of maize at Machakos

Treatment	Leaf Area (cm ²)	Stand Count	Height (cm)-80 DAP	Leaf No
Biogrovit	881a	15a	158.0a	17.5a
Biogrovit+Conventional	774b	16a	125.3b	11.0c
Control	436c	15a	93.8c	9.8c
Full conventional	858a	16a	128.0b	13.8b
SE	66.7	0.453	2.911	0.632
CV%	14.6	4.2	3	6.2
F pr.	<.001	0.287	<.001	<.001

115 Means followed by the same letter in each column are not significantly different at P≤0.05.
116 DAP-Days after planting

117 The increase in the growth components compared to the control might be due to the effect of
118 micro-organisms in the biofertilizer which colonized the plant and soil thus directly releasing
119 nutrients or by increasing availability of nutrients in the soils to plants. This is in accordance
120 to Vessey [8] who reported that biofertilizers are defined substances which contain living
121 micro-organisms and when applied to seed, plant surfaces or soil, colonize the plant and
122 promote its growth by increasing the nutrient availability. Also, Ali *et al.* [9] reported that
123 application of plant growth promoting rhizobacteria increased plant height and biological
124 yield. This agrees with Zahir et al. [10] (2004) that *Azotobacter* and *Azospirillum* are the most
125 important plant growth promoting rhizobacteria which affect the growth and development of
126 crops. Vessey, [8] posited that *Azotobacter* and *Azospirillum* enhance crop growth conditions
127 through several mechanisms especially through growth hormone production and improving
128 the efficiency of roots. Such growth promoting effect was maximal in response to inoculation
129 with mixture of *Azotobacter*, *Azospirillum* and PSM for all the growth parameters when
130 compared with the control. Improved plant growth by *Azospirillum sp* has been attributed to
131 both production of plant hormones, especially growth promoters, and by supplying combined
132 nitrogen [11].

133 3.2 Yield components

134 The application of treatments positively and notably influenced the grain yield and yield
135 components of maize in both sites (Table 3 and 4). The highest grain yield in Kirinyaga was
136 recorded under the biofertilizer treatment (8.60 t/ha) which however, did not differ
137 significantly with that under the conventional fertilizer treatment (7.55 t/ha). The lowest grain
138 yield was observed under the control (4.45 t/ha). The 1000-grain mass, number of cobs per

139 plant and the ear height were highest under the biofertilizer treatment, thus directly
 140 influencing the total grain yield in the end.

141 Table 3. Influence of test treatments on 1000-grain mass, number of cobs per plant, ear
 142 length and grain yield of maize at Kirinyaga

Treatment	1000-Grain weight (g)	Cobs/plant	Ear Length (cm)	Grain Yield (t/ha)
Biogrovit	351.5a	2.0a	15.9a	8.60a
Biogrovit+Conventional	260.8c	1.0c	11.4b	6.15b
Control	209.8d	1.0c	6.5c	4.45d
Full Conventional	299.5b	1.3b	13.2a	7.55a
SE	12.75	0.288	0.407	0.431
CV%	5.9	24.1	4.4	10.4
F pr.	<.001	<.001	<.001	<.001

143 Means followed by the same letter in each column are not significantly different at $P \leq 0.05$.

144 In Machakos, there were no significant differences between the biofertilizer and conventional
 145 fertilizer treatments which were however significantly higher than those under the
 146 combination of the biofertilizer and the conventional fertilizer, and the untreated control. A
 147 maximum of 7.77 t/ha was recorded under the biofertilizer treatment and 6.87 t/ha under the
 148 conventional fertilizer. The ear height, 1000-grain mass and number of cobs per plant are
 149 important traits that significantly differed due to the treatments with the highest recorded
 150 under the biofertilizer and conventional fertilizer treatments individually.

151
 152 Table 4. Influence of test treatments on 1000-grain mass, number of cobs per plant, ear
 153 length and grain yield of maize at Machakos

Treatment	1000-Grain weight (g)	Cobs/Plant	Ear Height (cm)	Grain Yield (t/ha)
Biogrovit	335.6a	1.78a	23.8a	7.77a
Biogrovit+Conventional	278.1b	1.13b	17.7b	5.95b
Control	198.7c	1.01b	13.0c	5.38d
Full Convectional	329.5a	1.13b	18.5b	6.87a
Standard error	12.75	0.0537	0.805	0.287
CV%	5.9	5.5	5.7	7.9
F pr.	<.001	<.001	<.001	<.001

154 Means followed by the same letter in each column are not significantly different at $P \leq 0.05$.

155 The positive effect of biofertilizer may have resulted from its ability to increase the availability
 156 of phosphorus and other nutrients especially under the influence of the calcareous nature of
 157 the soil, which cause decrease in the nutrients availability. These results agree with [12].
 158 Some researchers have also determined that enhanced phosphorus release increases
 159 evaluations for the trait of grain yield, biomass yield and 100-seed weight [13]. The 1000
 160 grain weight increases due to better transfer of photosynthetic substances under the
 161 biofertilizer treatments. It may be concluded that the photosynthetic capacity of plants treated
 162 with phosphorus-solubilizing micro-organisms increases due to increased supply of
 163 phosphorus nutrition. Cob weight increase may have been under the effect of the

164 phosphorus biofertilizer which induced the nutrient uptake ability of the roots and positively
165 increased the yield parameters because of improving the root system as a source-sink
166 relationship to the reproductive part (shoot), this agrees with [14]. Grain yield and biomass
167 yield increase were reported with the biofertilizer application which accounts important
168 benefits to the maize producers and maize production, causing a decrease in the inputs of
169 production because of economizing money compared to chemical fertilizers in order to
170 increase grain yield and biological yield. Biomass yield increased under application of
171 biofertilizers, because there was a significant increase in the dry weight of shoots at the
172 pretilking stage, that may be related to the favouritism of some environmental factors which
173 directly affected the bio fertilizer and its impact on the nutrient availability and growth, which
174 positively influenced maize photosynthesis and dry matter accumulation more actively that
175 agree with [15, 16]. Azimi et al [17] found that application of Supernitroplus biofertilizer with
176 Phosphate (Barvar 2) treatment had the highest seed yield (7.6 ton/ha) and non-application
177 of biofertilizers treatment that had Pishtaz cultivar had the lowest seed yield (6.3 ton/ha) [18].
178 Also, Azimi et al [2013b] found that the application nitrogen and phosphate biofertilizers
179 increased yield and yield components of barley under Boroujerd environmental condition
180 [18]. They suggested that grain yield and biomass yield increase was reported with the
181 biofertilizer application which is seen to be beneficial, causing a decrease in the production
182 costs because of spending less money compared to chemical fertilizers as mentioned earlier
183 [19].

184 The reduction in yield in the combined treatment of biofertilizer and conventional fertilizer
185 might be due to an antagonistic effect on plants and soils. The symbiotic association of
186 micro-organisms with plant roots is one of the most enhanced biological activities in the soil.
187 The neglectful interference of human activities such as over-application of fungicides and
188 frequent chemical phosphorus and nitrogenous fertilizer application has seriously threatened
189 this advantageous symbiosis as is in this case.

190 **4. CONCLUSION**

191 Application of biofertilizers is essential in the production of maize and therefore
192 recommended for its proper use is an environmentally friendly way of strengthening plant
193 growth and improvement.

194 **COMPETING INTERESTS**

195 Authors have declared that no competing interests exist.

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