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Testing of Commercial Inoculants to Enhance P Uptake and Grain Yield of Promiscuous Soybean in Kenya

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Abstract: The aim of this study was to assess the potential of commercial mycorrhizal inoculants and a rhizobial inoculant to improve soybean yield in Kenya. A promiscuous soybean variety was grown in a greenhouse pot study with two representative soils amended with either water-soluble mineral P or rock P to assess product performance. The performance of selected mycorrhizal inoculants combined with a rhizobial inoculant (Legumefix) was then assessed with farmer groups in three agroecological zones using a small-plot, randomized complete block design to assess soybean root colonization by mycorrhiza, nodulation, and plant biomass production in comparison to rhizobial inoculant alone or with water-soluble mineral P. Greenhouse results showed highly significant root colonization by commercial mycorrhizal inoculant alone ($p < 0.001$) and in interaction with soil type ($p < 0.0001$) and P source ($p < 0.0001$). However, no significant effect was shown in plant P uptake, biomass production, or leaf chlorophyll index. In field conditions, the effects of mycorrhizal and rhizobial inoculants in combination or alone were highly context-specific and may induce either a significant increase or decrease in root mycorrhizal colonization and nodule formation. Mycorrhizal and rhizobial inoculants in combination or alone had limited effect on plant P uptake, biomass production, leaf chlorophyll index, and grain yield. Though some mycorrhizal inoculants induced significant root colonization by mycorrhizal inoculants, this did not lead to higher soybean yield, even in soils with limited P content. Our results are further evidence that inoculant type, soil type, and P source are critical factors to evaluate commercial inoculants on a context-specific basis. However, our results highlight the need for the identification of additional targeting criteria, as inoculant

type, soil type, and P source alone were not enough to be predictive of the response. Without the identification of predictive criteria for improved targeting, the economic use of such inoculants will remain elusive.

Keywords: mycorrhizal inoculant; rhizobial inoculant; arbuscular mycorrhizal fungi; phosphorus; soil health

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is a rich source of plant protein (411 g kg^{-1}) and vegetable oil (209 g kg^{-1}). It is also used in a wide range of industrial products, from hand lotions to biofuel [1,2]. Soybean is a popular crop globally and its production and use in Kenya is gaining importance. However, compared to countries like Brazil (4 t ha^{-1}) [3], soybean yield in Kenya is relatively low (< 0.5 t ha^{-1}). The main reasons for low crop yields include poor soil fertility associated with poor organic content as a consequence of little or no nutrient inputs (both organic and inorganic) and P-deficient soils [4,5]. East African soils are highly deficient in P [6]. A recent comprehensive analysis [7,8] to understand the major limitations and opportunities to enhance soil fertility in sub-Saharan Africa, including Kenya, found that financially constrained farmers have limited incentive to use inorganic fertilizer alone or improved varieties without some improvement to soil health. Additionally, soybean is poised to dominate crop production across Africa both in terms of the expansion of harvest area and yield increases [9]. Thus, systems approaches that integrate in situ soil fertility enhancing technologies are of upmost prioritization to improve the productivity of crops. As such, soil microbials that enhance nutrient pools and uptake efficiency are likely technologies that can be valuable.

Soil microorganisms are comprised of a large pool of genetic diversity that plays a key role in soil processes, including nutrient cycling and acquisition [10]. Biological Nitrogen Fixation (BNF) with legumes is an important component of cropping systems in Africa. Efficient BNF requires the presence of efficient and compatible rhizobial strains in the soil (i.e., native or inoculated). This process can deposit large amounts of N into soils, thereby increasing plant yield [11,12]. Legume–rhizobia symbiosis has led to important economic benefits in terms of N-fertilizer savings of over USD $2.5 billion $year^{-1}$ [13] in Brazil and yields of above 2 t ha^{-1} in Zimbabwe [14]. In Kenya, the inoculation of a promiscuous soybean variety (TGx1740-2F) with commercial rhizobial inoculants (e.g., Legumefix) significantly enhanced N fixation and contributed to plant N nutrition under greenhouse conditions [15]. However, without enough P in the soil, BNF is limited [16].

Arbuscular mycorrhizal fungi (AMF) are a group of soil microorganisms that establish symbioses with most plant species. It has been widely reported [17,18] that AMF can play an important role in improving plant P nutrition and the nutrient status of other poorly mobile nutrients. This is primarily driven by AMF's extensive hyphal networks in soil [19,20] and the capacity of AMF to release organic acid to solubilize less soluble phosphate (i.e., rock phosphate) or other minerals fixed by soil components. AMF can acidify the soil through releasing H^+ or HCO_3^- ions that facilitate the transformation of HPO_4^{2-} into $H_2PO_4^-$, which is easily assimilated by plants [17]. Mycorrhiza are expected to play a key role in overcoming the exhausting of non-renewable P resources [21,22]. The efficient use of AMF could, in the near future, replace or reduce the quantitative use of chemical fertilizers, particularly phosphorus [23]. Inoculation with AMF can significantly increase the concentration of various macro and micro-nutrients, thus resulting in an increase in photosynthate production and therefore an increased accumulation of biomass [24,25].

Most commercial mycorrhizal inoculants contain both endo- and ectomycorrhiza species, which are occasionally mixed with beneficial bacteria and other ingredients. In addition, the quality of commercial AMF inoculants is sensitive to subtle changes in processing and transportation. Faye et al. (2013) tested twelve commercial mycorrhizal inoculants available on international markets and found

that only three were able to significantly improve root colonization levels compared to non-inoculated plants [26]. As suggested by Faye et al. (2013), commercial mycorrhizal inoculants should be pre-evaluated on selected crops and regional soils before launching large-scale field use to ensure the best fit of inoculants with conditions [26]. Currently, the potential of AMF to enhance plant growth and yield in field conditions is controversial. Numerous studies describe how AMF improved crop growth in greenhouse conditions [26–28]; however, none of these studies showed consistent effects in field conditions. Smith and Smith (2011) outlined factors that can explain the absence of improved crop productivity in the field due to AMF [29]. One such essential factor is the use of plant material from breeding programs (e.g., promiscuous soybean varieties). A meta-analysis was conducted (i.e., 1981 to 2010, 39 publications working on 320 different crop plant genotypes) about mycorrhizal responsiveness trends in annual crops and their wild relatives [30]. Their conclusion was that new cultivars were more mycorrhiza-responsive and possibly dependent compared to ancestral genotypes. In addition, in cases of a large presence of adapted native AMF, field inoculations with exotic commercial mycorrhizal inoculants were not successful.

There are several examples that highlight the benefits of dual inoculation with both AMF and rhizobia by enhancing plant growth [31–33]. However, to the best of our knowledge, such a dual inoculant in a single product is not commercially available and the performance of such a product would likely be dependent on product composition and environmental conditions, making it difficult to generalize. The aim of this study was to assess the potential of commercial mycorrhizal inoculants and a rhizobial inoculant to improve soybean yield in Kenya. We hypothesize that the performance of commercially available inoculants will either increase or decrease as a consequence of P and N source and soil type, and that a dual inoculation of mycorrhiza–rhizobia will enhance plant tissue P and N status (i.e., as indicated by leaf chlorophyll index), and consequent biomass and grain yield, in each site.

2. Materials and Methods

Greenhouse Experiment

A pot experiment was conducted in a greenhouse at the Tropical Soil Biology and Fertility (TSBF) institute of the Centre International d'Agriculture Tropicale (CIAT), Nairobi, Kenya using two representative soils from central and coastal provinces of Kenya. The soils were classified as Humic Nitisols and Rhodic Ferralsols, respectively, according to the World Reference Base for soil resources [34]. Soils selected are responsive to both N and P fertilizer and contained a low to moderate level of organic carbon with an acidic pH near to neutral. At the time of soil collection both fields were cultivated with maize without the previous application of fertilizer or organic inputs, and had never been cultivated with soybean. Soils were collected from the 0-20 cm soil layer, shade-dried, sieved to pass through 4-mm holes, homogenized, and analyzed. The key characteristics of the soil are presented in Table 1.

The number of indigenous mycorrhizal spores were quantified for both soils. AMF spores were extracted from a 100 g sample by wet sieving and sucrose gradation [35]. An Asian soybean genotype, bred for promiscuous nodulation from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria [36] was used in this research. This soybean variety is described as widely adapted and resistant to major diseases in Kenya [15]. Pots were filled with 2.3 kg of the central province soil and 2.4 kg of the coastal province soil due to differences in bulk density and arranged according to a randomized complete block design (RCBD) with three replicates. Prior to planting, seeds were surface-sterilized using 3.3% $Ca(OCl)_2$ for five minutes and rinsed five times with sterile distilled water. To avoid nutrient deficiency, which could affect crop growth, all nutrients except for P were applied at optimal rates as follows: KNO_3 (0.562 g kg^{-1} of dry soil), $MgSO_4$ (0.133 g kg^{-1}), $CaCl_2$ (0.287 g kg^{-1}), and $MgCl_2$ (0.290 g kg^{-1}). These calculations were based on optimal foliar concentrations and an expected maximal biomass yield of 60 g dry matter (DM) $plant^{-1}$. Nutrient solutions were prepared and thoroughly

mixed with the soil, prior to planting. P was provided as water-soluble mineral P (KH_2PO_4) at 1/5 phosphate sorption indices (i.e., 2.7 mg P kg^{-1} of soil for the central province soil and 63 mg P kg^{-1} of soil for the coastal province soil both targeting a P concentration of 0.2 mg P L^{-1} in solution [37]) or as Minjungu Rock Phosphate (Minjungu PR) (25% of P_2O_5) at 60 kg ha^{-1}. Pots were rotated daily and soil moisture content was brought to 18% and 27% (w/w) for the coastal and central province soils, respectively (i.e., approximately 90% of soil field capacity) by weighing and adding distilled water.

Table 1. Characteristics of the Kenyan soils used for the greenhouse study.

Soil Characteristics	Coastal Sandy Soil (Kilifi)	Central Clay Soil (Chuka)
pH (1:2.5 soil: H_2O)	7.30	5.83
Organic C (%) *	0.98	2.63
Total N (%) **	0.08	0.23
Olsen P (mg P kg^{-1} soil)	18.3	4.27
Exchangeable K (cmol kg^{-1}) ***	0.64	1.73
Exchangeable Ca (cmol kg^{-1}) ***	9.48	12.7
Exchangeable Mg (cmol kg^{-1}) ***	3.42	3.28
Water holding capacity (%) $^{\beta}$	18.7	29.5
Indigenous AMF spore number (100g dry $soil^{-1}$)	10.7	12
Sand (%) §	78	53
Silt (%) §	10	24
Clay (%) §	12	23
Texture class	Sandy loam	Sandy clay-loam
Soil class	Rhodic Ferralsol	Humic Nitisol

All methods follow [4]; * determined by sulfuric acid and aqueous potassium dichromate ($K_2Cr_2O_7$) mixture, ** hydrogen peroxide + sulfuric acid + selenium and salicylic acid digestion, *** extracted with 1 M NH_4OAc (ammonium acetate) solution, $^{\beta}$ field water holding capacity method, § hydrometer method.

The commercial rhizobial inoculant Legumefix was obtained from Legume Technology (UK), as it performed well with the promiscuous soybean variety TGx1740-2F under controlled conditions [15]. The twelve commercial mycorrhizal inoculants were obtained from Agrauxine (France), IFTECH (France), Mycorrhizal Applications Inc. (USA), Dudutech Ltd. (Kenya), Nutri-Tech Solutions (Australia) and Zander Middle East (Emirate). Endorhize standard, Endorhize premium, Mycor, and Vam-Tech declared to have a single strain of AMF, whereas, Myco Apply Endo and Rhizatech declared to contain multiple strains of AMF. Myco Apply Maxx, Myco Apply Endo plus, Myco Apply Soluble Endo, Myco Apply Root Dip Gel, and Myco Apply Soluble Soluble Maxx declared to contain endomycorrhiza (*Glomus*, *Gigaspora*), ectomycorrhiza (*Pisolithus*), and several kinds of other fungi and bacteria such as *Trichoderma* or *Bacillus* (Table 2). For Zander Mycorrhiza, the manufacturer did not provide species information about the content of the inoculants. These inoculants were selected using scientific judgement to cover a diversity of species including mycorrhiza. Many of the products were commercially available but not necessarily available in Kenya. Thus, the tested inoculants represented a fairly exhaustive list of commercially available products. All commercial inoculants were applied according to the manufacturer's protocol (i.e., as seed coating, blending into potting soil, drench or soil application, or dipping root into product) and were used before the expiration date. Average minimum and maximum temperatures in the greenhouse during plant growth were 18 °C and 38 °C, respectively. For the six-week growing period, plant shoot heights were measured twice a week. Chlorophyll index was determined in the three youngest fully developed leaves at four and six weeks after planting using a chlorophyll meter (SPAD 502; Konica Minolta Sensitive, Inc; 101 Williams Drive Ramsey, NJ, USA).

Table 2. List and characteristics of tested commercial arbuscular mycorrhizal fungi (AMF) inoculants.

Product Name /Producer	Asserted Active Ingredients
(In. 1) Endorize standard /Agrauxine (France)	*Glomus* spp
(In. 2) Endorize premium/Agrauxine (France)	*G.* spp
(In. 3) Myco Apply Endo/Mycorrhizal Applications, Inc. (USA)	*G. intraradices*, *G. mosseae*, *G. aggregatum* and *G. Etunicatum*
(In. 4) Myco Apply Endo plus /Mycorrhizal Applications, Inc. (USA)	*G. intraradices*, *G. mosseae*, *G. aggregatum and G. etunicatum*,*Trichoderma konigii* and *T. harzianum*
(In. 5) Myco Apply Maxx /Mycorrhizal Applications, Inc. (USA)	*G. intraradices*, *G. mosseae*, *G. aggregatum*, *G. etunicatum*, *Rhizopogon villosullus*, *R. luteolus*, *R. amyopogon*, *R. fulvigleba*, *Pisolithus tinctorius*, *Scleroderma ceap* and *S. citrinum T. koningii* and *T. harzianum*, *Bacillus licheniformis*, *B. pumilis*, *B. amyloliquefaciens* and *B. megaterium*
(In. 6) Myco Apply Soluble Endo /Mycorrhizal Applications, Inc. (USA)	*G. intraradices*, *G. mosseae*, *G. aggregatum* and *G. etunicatum*
(In. 7) Myco Apply Root Dip Gel /Mycorrhizal Applications, Inc. (USA)	*G. intraradices*, *G. mosseae*, *G. aggregatum*, *G. monosporum*, *G. cralum*, *G. deserticola*, *Gi. margarita*, *Gi. brasilianum*, *Gi. etunicatum*, *R. vilosullus*, *R. lutelolus*, *R. amylopogon*, *R. fulvigleba*, *Pisolithus tinctorius*, *Scleroderma Cepa*, *S. cirtrinum propagules.*
(In. 8) Myco Apply Soluble Maxx /Mycorrhizal Applications, Inc. (USA)	*G. intraradices*, *G. mosseae*, *G. aggregatum*, *G. etunicatum G. clarum*, *G. deserticola*, *Gi. margarita*, *Gi. brasilianum*, *Gi. monosporum*, *R. villosullus*, *R. luteolus*, *R. amylopogon*, *R. fulvigleba*, *Pisolithus tictorius*, *Laccaria bicolr* and *L. laccata*, *Scleroderma cepa* and *Sc. citrinum*, *Suillus granulates* and *Su. puctatapies*, *T. harzin* and *T. konigii*, *Bacillus licheniformis*, *B. azotoformans*, *B. megaterium*, *B. coagulans*, *B. pumilis*, *B. thuringiensis*, *B. stearothermiphilis*, *Paenibacillus polymyxa*, *Pa. durum*, *Pa. florescecne*, *Pa. gordonae*, *Azotobacter polymyxa*, *Az. chroococcum*, *Sacchromyces cervisiae*, *Pseudomonas aureofaceans*
(In. 9) Mycor/Iftech (France)	*G. intraradices*
(In. 10) Rhizatech /Dudutech (K) Ltd. (Kenya)	Spores and mycelial fragments of AMF (mainly *G. intraradices*)
(In. 11) Vam-Tech /Nutri-Tech Solutions P/L (Australia)	*Glomus intraradices*
(In. 12) Zander Mycorrhiza /Zander Middle East LLC, United Arab Emirates	Beneficial arbuscular mycorrhizal fungi from arid zones

At 42 days after planting, plants were harvested by cutting the stem one cm above the soil surface. Pot contents were washed over a two-millimeter sieve to isolate roots and nodules, which were counted, weighed, and stored in glycerol at −20 °C. Shoot biomass samples were oven dried for 72 hours at 65 °C and dry biomass was recorded before grinding and quantifying P tissue content at the TSBF CIAT labs. Roots were kept in ethanol (70%) at 4 °C for further mycorrhizal root colonization assessment in each pot treatment by staining with Trypan blue [38] and the percent root colonization was determined using the magnified intersections method [39]. This is an objective scale of measurement, involving the inspection of arbuscules intersecting the vertical crosshair at a magnification ×200. Treatment effects and interactions (i.e., P uptake, plant above ground biomass, chlorophyll index, fresh nodule biomass, and root colonization) were assessed using the PROC mixed procedure of the SAS program [40] and significance was evaluated at $p \leq 0.05$ according to Tukey's test.

3. Field Experiment

Experimental Sites, Design, and Management

The best-performing Mycorrhizal inoculants from the greenhouse study were selected for inclusion in the field trial in combination with the rhizobial inoculant Legumefix alone or with two P sources in a multi-location trial during the rainy season of August–December 2010 in three different agroecological zones (AEZ) of Kenya (Figure 1).

Figure 1. Map of the agroecological zones where the commercial mycorrhizal inoculants were evaluated (i.e., Bungoma, Bondo, and Chuka) and where the clay and sandy soils used in greenhouse experiments were collected (i.e., Chuka and Kilifi).

Sites were selected to cover a gradient of rainfall, altitude, and temperature. In each AEZ, three farmers from seven representative farmers' groups were selected (i.e., 3 × 7 = 21 farms and considered each as a replicate) based on a homogeneous cropping history and previous field responsiveness to N and P fertilizers. Poor, unused or extremely fertile sites and sites on steep slopes were avoided. Soils were sampled and characterized before trial initiation (Table 3). Peat-based inoculants were applied to the seeds at triple the rate of the on-field recommended dose and converted using the optimal planting density (i.e., 270,000 plants ha^{-1}) to ensure enough propagules. Within each farm, plots of 22.5 m^2 were separated by 1 m alleys and were laid out according to an RCBD. Soybean plants were spaced with 0.75 m between rows and 0.05 m within rows. The selected mycorrhizal inoculants from the greenhouse study (i.e., Endorize premium, Myco Apply Maxx and Rhizatech) were applied in combination with a rhizobial inoculant (i.e., Legumefix) and compared to a reference treatment (i.e., Legumefix alone and Legumefix + diammonium phosphate (DAP) at recommended rate (50 kg ha^{-1})).

Table 3. Physicochemical properties of agroecological soils of Bondo, Bungoma, and Chuka before trial initiation.

	Sites		
Soil Characteristics	**Bondo**	**Bungoma**	**Chuka**
Soil pH (1:2.5 soil: H_2O)	6.48	5.26	5.60
Organic C (%) *	2.71	1.04	3.30
Total N (%) **	0.12	0.08	0.92
Olsen P (mg P kg^{-1} soil)	8.00	9.63	11.27
Exchangeable K (cmol kg^{-1}) ***	2.99	0.29	1.07
Exchangeable Ca (cmol kg^{-1}) ***	8.73	2.75	3.12
Exchangeable Mg (cmol kg^{-1}) ***	0.84	0.77	9.45
Cation exchange capacity (cmol kg^{-1}) $^{\beta}$	15.1	7	21.35
Indigenous AMF spores number (soil g^{-1})	8.3	8	6.5
Clay (%) §	59	20	45
Sand (%) §	11	67	15
Silt (%) §	30	13	40
Textural Class	Sandy clay loam	Loamy sand	Sandy clay
Soil Class	Vertisol	Orthic Ferralsol	Rhodic Nitisol

All methods follow [4]; * determined by sulfuric acid and aqueous potassium dichromate ($K_2Cr_2O_7$) mixture, ** hydrogen peroxide + sulfuric acid + selenium and salicylic acid digestion, *** extracted with 1 M NH_4OAc (ammonium acetate) solution, $^{\beta}$ exchangeable acidity by the titration method, § hydrometer method.

Treatment effects on soybean biomass production, the nodule fresh weight (FW) of the plant^{-1}, and root colonization by mycorrhiza were assessed at four weeks after planting on five randomly selected plants at the 50% podding stage (i.e., R4) [41] along a 1-m transect within each plot. Root colonization by mycorrhiza and percent root colonization were measured using the same method as conducted in the greenhouse study [38,39]. Grain yield was evaluated at harvest from 50 soybean plants in each plot and yield (t ha^{-1}) was extrapolated according to the following formula:

$$\text{Yield ha}^{-1} = \frac{\text{SSPDW} \times \text{TOTPFW}}{\text{SSPFW} \times \text{HNP}} \times 10 \quad (1)$$

Sub-Sample Pod Dry Weight (SSPDW); Sub-Sample Pod Fresh Weight (SSPFW); Total Pod Fresh Weight (TOTPFW); Harvest Net Plot (HNP), and ×10 conversion factor from kg/m^2 to t/ha.

Considering each farm as a replicate, an analysis of variance (ANOVA) was conducted to determine the effects of inoculants across the three sites using a mixed linear model [40]. By computing least square means and standard errors of difference, the effects of the different treatments were compared. A mean comparison was performed using Fisher's protected least significant difference (LSD) test at $p \leq 0.05$ significance level.

4. Results

4.1. Greenhouse Experiment

4.1.1. Root Colonization and Nodulation

Plant root colonization by mycorrhiza was affected by inoculants ($p < 0.001$) as well as inoculant interactions with soil type and P source ($p < 0.0001$) (Table 4). Myco Apply Endo plus (In. 4) (51.2%), followed by Endorize premium (In. 2) (48.2%), and Rhizatech (In. 10) (40.3%) had significantly higher percent root colonization compared to control (Table 5). The nodule FW of the plant^{-1} was not affected by inoculant application or soil type. However, inoculants and P source significantly ($p = 0.0357$) improved plant nodulation (Table 4). There was no relationship between the application of AMF inoculants alone or their interaction with soil type on soybean plant nodulation (Table 5). When water-soluble mineral P was applied, two inoculants (i.e., Myco Apply Soluble Maxx (In. 8)

and Zander Mycorrhiza (In. 12)) reduced the number of nodules plant^{-1} compared to the control (i.e., 0.80 g plant^{-1} and 0.98 g plant^{-1}, respectively).

Table 4. Analysis of variance of commercial mycorrhizal inoculant effects on soybean growth, root colonization, nodulation, leave chlorophyll index and P uptake under greenhouse conditions.

Sources of Variation	Plant P Uptake	Root Colonization	Biomass Production	Nodules Fresh Weight	Leaf Chlorophyll Index
Inoculants alone	$p = 0.2787$	$p < 0.001$	$p = 0.6449$	$p = 0.560$	$p = 0.1356$
Inoculants-Soil Type	$p = 0.0926$	$p < 0.0001$	$p = 0.0557$	$p = 0.2877$	$p = 0.2709$
Inoculants-P Source	$p = 0.1559$	$p < 0.0001$	$p = 0.1188$	$p = 0.0357$	$p = 0.8580$

Commercial mycorrhizal inoculant interactions with soil type resulted in highly significant effects on soybean plant root colonization by AMF ($p < 0.0001$). For the control treatments without mycorrhizal inoculants, soybean root colonization was higher in the clay soil compared to the sandy soil (31.8% and 13%, respectively). In the sandy soil (i.e., coastal province), inoculants Myco Apply Root Dip Gel (In. 7) (13.8%) and Myco Apply Soluble Endo (In. 6) (18.5%) had similar effect on plant root colonization to the control (13.0%). In contrast, inoculants Endorize premium (In. 2) (40.5%), Zander Mycorrhiza (In. 12) (37.8%), Endorize standard (In. 1) (33.3%), Vam-Tech (In. 11) (32.5%), Myco Apply Maxx (In. 5) (31.8%), Mycor/Iftech (In. 9) (28.5%), Myco Apply Soluble Maxx (In. 8) (28.0%), Rhizatech (In. 10) (27.0%), and Myco Apply Endo (In. 3) (20.0%) significantly increased root colonization compared to non-inoculated plants. In the clay soil (i.e., central province), all inoculants increased plant root colonization by AMF inoculation. Myco Apply Endo plus (In. 4) (65.5%), Endorize premium (In. 2) (55.8%), and Rhizatech (In. 10) (53.7%) significantly increased root colonization compared to the control (31.8%).

In the presence of Minjungu PR, Myco Apply Endo plus (In. 4) (61.1%), Endorize premium (In. 2) (48.7%), Vam-Tech (In. 11) (38.5%), Zander Mycorrhiza (In. 12) (38.0%), and Rhizatech (In. 10) (33.7%) had a significant higher percent root colonization compared to the control (15.5%). All other inoculants did not statistically improve percent root colonization in the presence of Minjungu PR. When water-soluble mineral P was applied, the percentage root colonization by AMF was found to be similar to control plants (29.3%) except for Myco Apply Endo (In. 3), which had significantly lower (19.0%) root colonization. Endorize premium (In. 2), Rhizatech (In. 10), Mycor/Iftech (In. 9), Myco Apply Root Dip Gel (In. 7), and Myco Apply Endo plus (In. 4) had the highest percentage of root colonization by AMF (47.7%, 47%, 42.7%, 42.5%, and 40.8%, respectively), though this was not significantly different from the control.

4.1.2. P Uptake, Leaf Chlorophyll Index, and Biomass Production

In contrast, inoculants alone, when interacting with soil type or interacting with P source, did not significantly improve plant P uptake, biomass production, or chlorophyll index ($p \leq 0.05$) (Table 4).

Table 5. Effects of commercial mycorrhizal inoculants on soybean nodulation and root mycorrhizal colonization at 42 days after planting in greenhouse. Inoculant effects are those due to the product alone, without taking in account the soil and the P source.

	Nodules FW (g Plant^{-1})		Root Mycorrhizal Colonization (%)				
	Inoculant-P Source		Inoculant	Inoculant-Soil Type Interaction		Inoculant-P Source Interaction	
AMF Inoculants	**Rock P**	**Water Soluble Mineral P**		**Sandy Coastal Soil**	**Clay Central Soil**	**Rock P**	**Water Soluble Mineral P**
Control	1.38a	1.27ab	22.2d	13.0c	31.8de	15.5e	29.3ab
In. *1	1.81a	1.41ab	30.3cd	33.3abc	27.3de	26.7cde	34.0ab
In. 2	1.33a	1.19ab	48.2ab	40.5a	55.8ab	48.7ab	47.7a
In. 3	1.46a	1.56ab	30.0cd	20.0abc	22.7e	17.5de	19.0b
In. 4	1.21a	1.58ab	51.2 a	36.8ab	65.5a	61.1a	40.8ab
In. 5	1.50a	1.89a	30.8cd	31.8abc	29.8de	24.7cde	37.0ab
In. 6	0.92a	1.54ab	29.3cd	18.5bc	40.0bcde	26.5cde	32.0ab
In. 7	1.41a	1.78ab	21.3d	13.8c	46.2abcd	23.7cde	42.5ab
In. 8	1.31a	0.80a	31.3cd	28.0abc	34.7cde	24.8cde	37.8ab
In. 9	1.20a	1.36ab	33.4cd	28.5abc	38.3bcde	24.7cde	42.7ab
In. 10	1.46a	1.56ab	40.3abc	27.0abc	53.7abc	33.7bcd	47.0a
In. 11	2.05a	1.18ab	34.6bcd	32.5abc	36.7bcde	38.5bc	30.7ab
In. 12	1.54a	0.98ab	33.9bcd	37.8ab	30.0de	38.0bc	29.8ab

* = Mycorrhizal inoculant. Values are means of four replicates. According to Tukey's test, mean values followed by similar letters in the same column are not significantly different from each other at $p \leq 0.05$. Fresh weight (FW). Mean values followed by similar letters (a,b,c,d) in the same column are not significantly different from each other at $p \leq 0.05$.

4.2. Field Experiment

4.2.1. Plant Root Nodulation and AMF Colonization

In the greenhouse, Rhizatech (In. 10), Endorize Premium (In. 2), and Myco Apply Maxx (In. 5) all performed well and were thus selected for inclusion in the field trials. Additionally, P source had significant effect on root colonization and nodule formation with water-soluble mineral P performing better than Minjungu RP, thus, a water-soluble mineral P treatment in combination with Legumefix was included in the field trial. The results of the single or dual field inoculations with selected commercial inoculants on soybean plant nodulation and root colonization by AMF are summarized in Table 6. When Legumefix was inoculated alone, nodule biomass was 7.6 g plant^{-1} in Bondo and was poor compared to 18.4 g plant^{-1} and 28.9 g plant^{-1} in Bungoma and Chuka, respectively. In Chuka, the combination of Legumefix with the mycorrhizal inoculant Rhizatech was the only treatment to induce significantly higher nodule FW plant^{-1} (49.3 g plant^{-1}) across all combinations of P and mycorrhizal inoculants compared to Legumefix alone. However, this combination significantly decreased nodule plant^{-1} FW in Bungoma and Bondo. In general, besides the combination of Legumefix with the mycorrhizal inoculant Rhizatech in Chuka, the combination of Legumefix with DAP or any other commercial mycorrhizal inoculants significantly decreased nodule FW plant^{-1} across all sites (Table 6). Reduction in nodule FW was as severe as 5.4-fold decrease in Bondo.

Table 6. Field assessment of soybean root arbuscular mycorrhizal fungi (AMF) colonization and nodulation levels after the application of select commercial inoculants (Legumefix–rhizobia; Endorhize–AMF; Rhizatech–AMF; Myco Apply Maxx–microbial mixture) with or without diammonium phosphate (DAP) in the three agroecological zones.

Inoculations	Bungoma		Bondo		Chuka	
	Root AMF Colonization (%)	Nodule FW Plant^{-1}	Root AMF Colonization (%)	Nodule FW Plant^{-1}	Root AMF Colonization (%)	Nodule FW Plant^{-1}
Legumefix	37.8b	18.4a	21.6c	7.6a	6.7a	28.9b
Legumefix+ DAP	22.2c	11.4b	19.0c	1.4b	6.7a	11.6c
Endorize Premium (In. 2) +Legumefix	35.6b	14.6b	32.3b	3.3b	9.7a	27.5b
Rhizatech (In. 10) +Legumefix	53.3a	14.7b	41.8a	1.4b	8.7a	49.3a
Myco Apply Maxx (In. 5) +Legumefix	23.3c	17.4a	32.0b	2.1b	NA	NA

Values are means with a minimum of four replicates. Mean comparisons were performed by the Fisher's protected LSD test. Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$. Not assessed (NA). Fresh weight (FW) (g).

Legumefix with the mycorrhizal inoculant Rhizatech significantly increased mycorrhizal colonization percentages in Bungoma and Bondo (i.e., ~2X) but had no effect in Chuka, unlike in the case of nodule FW (Table 6). Endorize Premium and Myco Apply Maxx in combination with Legumefix increased mycorrhizal colonization percentage in Bondo only. Legumefix in combination with DAP significantly reduced the mycorrhizal colonization percentage in Bungoma and had no effect in Bondo and Chuka. The other mycorrhizal inoculants in combination with Legumefix either had no significant effect on mycorrhizal colonization percentage or induced a significant decrease in mycorrhizal colonization percentage. Treatment effects varied significantly across locations for both nodule FW and mycorrhizal colonization percentage.

4.2.2. Soybean Yield

Despite higher root AMF colonization percentages and fresh nodule biomasses induced by several dual inoculations with both mycorrhizal and rhizobial inoculants in specific locations (Table 6), soybean yield was not statistically enhanced regardless of location (Table 7). There were also no differences in yield between treatments. Soybean grain yields ha^{-1} were on average higher in Bondo, followed by Chuka and Bungoma ($p \leq 0.05$) but were relatively low with a maximum average yield of 0.75 t ha^{-1} in Bondo. The control (Legumefix alone) ranged from 0.71 t ha^{-1} in Chuka to 0.46 t ha^{-1} in Bungoma.

DAP applications combined with Legumefix did not significantly increase soybean grain yield (0.62 t ha^{-1}, 0.82 ha^{-1}, and 0.57 t ha^{-1} in Bungoma, Bondo, and Chuka, respectively).

Table 7. Field assessment of soybean yield after application of the selected commercial inoculants (Legumefix–rhizobia; Endorhize–AMF; Rhizatech–AMF; Myco Apply Maxx–microbial mixture) with or without DAP in three agroecological zones.

	Grain Yield (t ha^{-1})		
Inoculation	**Bungoma**	**Bondo**	**Chuka**
Legumefix	0.46a	0.69a	0.71a
Control (Legumefix + DAP)	0.62a	0.82a	0.57a
Endorize premium (In. 2) + Legumefix	0.53a	0.76a	NA
Rhizatech (In. 10) + Legumefix	0.65a	0.64a	0.61a
Myco Apply Maxx (In. 5) + Legumefix	0.53a	0.83a	NA

Values are means with a minimum of four replicates. Mean comparisons were performed by the Fisher's protected LSD test. Values followed by the same letter in the same column are not significantly different at $p \leq 0.05$. Not assessed (NA).

5. Discussion

Inoculant type, soil type, and P source are critical factors to evaluate commercial inoculants on a context-specific basis [42–44]. The best plant root colonization responses appeared in the presence of water-soluble mineral P for nine of the twelve inoculants (Table 5), which can be explained by the quick availability of this form as opposed to Minjungu RP, which needs more time to solubilize. Nodule formation is a P-demanding process [43,44] and the faster solubilizing mineral P source likely accounts for this observation, while improved P uptake due to AMF begins with the colonization of plant roots [17,45], which should have increased P uptake with increased mycorrhizal colonization; however, this was not observed. Enhanced P uptake is generally regarded as one of the most important benefits that AMF provides to host plant [20,46]. In both the greenhouse and the field, none of the products significantly improved plant P uptake. Though this study does not shed light on the effect of commercial inoculants on the uptake of other essential nutrients, it is worth noting that AMF can deliver nutrients other than P such as Zn, Ca, Cu, K, S and N [16]. Additionally, since a complete nutrient package was applied in the greenhouse but not in the field conditions, it may be theorized that the total soil nutrient balance is important for the success of mycorrhizal inoculation. The difference in results from the greenhouse to the field could also be related to niche and nutrient competition with indigenous strains, as reported in other studies [44,47].

In general, Rhizatech, which is a local, Kenyan-sourced inoculant, performed best in terms of inducing root mycorrhizal colonization and nodule formation, though not consistently across locations. This result contradicts others that have shown that the inoculation of maize by exotic AMF strains favored higher root colonization than that by the native AMF strains [48]. These authors also believe that, except for the case of strains like *Gigaspora margarita*, the use of colonization stimulants may be necessary to increase the mycorrhizal colonization of the roots. However, even though Rhizatech induced high root infection, this did not translate into improved plant growth efficiency. Myco Apply Maxx mitigated root colonization in some AEZ (Chuka) and significantly improved promiscuous soybean plant nodulation in other AEZ (Bungoma) highlighting the highly variable response of some inoculants. Others have suggested that mixing inoculants may exploit their synergetic effects [49,50]; however, Myco Apply Max had very inconsistent results even though it is reported to contain nine AMF species, 15 bacterial species, 11 *Ectomycorrhiza* strains, two *Trichoderma* strains, and NPK (4-1-2). Microbial components compete for establishment and food and thus the multiple species competing in the inoculant mix may have contributed to its lack of effect [51]. Inhibitory effects between inoculant ingredients, soil microorganisms, and soil components may determine the success of mycorrhizal field inoculation and should be further investigated. Plant root colonization by mycorrhiza is also determined by various factors including the presence and quality of indigenous populations. Foreign

strain inoculants often fail to form mycorrhizal associations [28,52] and this likely accounts for our results showing that three inoculants significantly increased soybean root mycorrhizal colonization, whereas nine other inoculants were not significantly different (Table 4).

We also observed a competition for colonization between the native strains of AMF and the exotic strains contained in the inoculants. Plant roots were shown to be colonized simultaneously by more than one AMF strain in both natural and agricultural ecosystems, which is consistent with other studies [17,53]. The number of AMF spores found in the soils used at the greenhouse and in the field indicated that the populations of native AMF were not negligible (around 1135 spores per 100 g of dry soil and 770 spores per 100 g of dry soil, respectively). Molecular techniques would be needed to distinguish between native and inoculated mycorrhiza strains [53], which was not conducted for this study. Moreover, others have shown that despite a high number of nodules occupied by the inoculated rhizobial strain of Legumefix on the promiscuous soybean variety TGx1740-2F, this did not lead to an increase in grain yield [54]. According to these authors, soil factors (e.g., pH, P, C, N) seemed to affect which strain would occupy the nodule. Accordingly, the same assumption could be made regarding the efficiency of the root mycorrhizal symbiosis induced by AMF. Additionally, in the greenhouse, percent root colonization by mycorrhiza was higher with or without commercial inoculants on clay soil. In the field conditions, however, clay content did not appear to be the primary factor determining increased root colonization by mycorrhiza as Bungoma had the lowest clay content but had significantly higher percent root colonization by mycorrhiza compared to the locations with greater clay content than the greenhouse clay soil. Independent to soil type or P source, most of the tested commercial mycorrhizal inoculants from this study improved promiscuous soybean root colonization by mycorrhiza, which is further evidence that new plant genotypes have maintained susceptibility to mycorrhizal inoculation [30]. Using many of the same commercial inoculants, our results also seem to suggest that soybean response to these inoculants is better than with maize [26].

6. Conclusions

Regardless of whether they were tested in greenhouse or field conditions, the effects of mycorrhizal and rhizobial inoculants in combination or alone are highly context-specific and may induce either a significant increase or decrease in root mycorrhizal colonization and nodule formation. Mycorrhizal and rhizobial inoculants in combination or alone had limited effect on plant P uptake, biomass production, leaf chlorophyll index, and grain yield. Additionally, though some inoculants induced significant root colonization by AMF, this did not lead to higher soybean yield, even in soils with limited P content, as in the case of Kilifi and Chuka. Location- and management- related factors such as soil type and P application source had significant treatment effects in both the greenhouse and field conditions. For the Chuka soil, percent root colonization by mycorrhiza was greater in the greenhouse than in field conditions across all inoculants. Our results are further evidence that inoculant type, soil type, and P source are critical factors to evaluate commercial inoculants on a context-specific basis. However, our results highlight the need for the identification of additional targeting criteria, as these three factors alone were not enough to be predictive of a response. Without the identification of predictive criteria for improved targeting, the economic use of such inoculants will remain elusive.

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