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Full Length Research Paper

Evaluation of microbial inoculants as biofertilizers for the improvement of growth and yield of soybean and maize crops in savanna soils

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Certain rhizobacteria exert considerable influence on plant growth and development, particularly under limiting conditions. The effects of some indigenous soil microbial isolates and commercially produced microbial inoculants, referred to as bio-inoculants, on the growth and dry matter yield (DMY) of maize (*Zea mays*) and soybean (*Glycine max*) crops were assessed under greenhouse conditions. In two sets of experiments, one set comprised of free-living nitrogen-fixing microorganisms (*Azospirillum* spp.), three soils from Ibadan, Mokwa and Shanono located in different agro-ecological zones, and maize as the test crop. The other set consisted of microbial inoculants that can act as biocontrol agents applied to sterilized and non-sterilized soils; soybean was the test crop. The bio-inoculants were applied separately and also in combination. The treatments included a reference termed 'mineral N' where macro- and micro-nutrients were supplied at optimal rates, a control where bio-inoculants were not applied, and four replicates. All treatments, excluding the reference, received only macro-nutrients at suboptimal rates. The crops were grown for eight weeks and growth parameters were measured. The shoot DMY of maize was relatively large (42 to 63 g plant^{-1}) and differed significantly among the soils but the bio-inoculants did not improve the shoot DMY significantly ($P > 0.05$) in any of the soils when compared with the control. However, sole inoculation of Mazospiriflo-2 enhanced nitrogen uptake significantly in maize grown in Shanono soil. For soybean, the shoot DMY was also not improved by the inoculation or the addition of the microbial products compared with the control.

Key words: Biocontrol, cereals and legumes, inoculum, plant growth promoting rhizobacteria, soil microorganisms.

INTRODUCTION

Crop production needs to be increased substantially to reduce hunger and food insecurity in West Africa. Since most soils in the region are inherently poor, external inputs such as mineral or organic fertilizers are necessary to boost crop production. However, marginal farmers do not use sufficient amounts of mineral fertilizers for various reasons (Manyong et al., 2001). In addition, there is a need to improve crop productivity in an ecofriendly manner and this has led to the promotion of commercial biological and chemical products intended to restore or enhance the fertility and organic matter content of soils. Soil microorganisms play significant role in organic matter decomposition and release of plant nutrients such as nitrogen (N), phosphorus (P) and sulfur (S). Therefore, microorganisms are important component of integrated nutrient management systems and soil biodiversity. It is assumed that many of the biological products being offered to farmers contain beneficial microorganisms (bacteria and fungi) with the potential to promote plant

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Table 1. Selected properties of the soils used for the evaluation.

Parameter	Soil		
	Mokwa	Shanono	Ibadan
pH (H_2O)	5.8	6.3	4.6
Organic C (g kg^{-1})	5.7	4.2	6.1
N (g kg^{-1})	0.37	0.35	0.52
Extractable P (Melhlich, mg kg^{-1})	2.94	14.03	10.53
Mg (cmol kg^{-1})	0.39	1.18	0.22
K (cmol kg^{-1})	0.17	0.38	0.14
Zn (mg kg^{-1})	1.34	0.83	5.64
Sand (g kg^{-1})	710	750	760
Silt (g kg^{-1})	150	120	120
Clay (g kg^{-1})	140	130	140

health. Rhizobia are bacteria that fix atmospheric N, a process referred to as biological N fixation (BNF), in the nodules formed by the roots of leguminous plants. It is estimated that rhizobia can fix about 50 to 300 kg N ha^{-1} (Bokhtiar and Sakurai, 2005). Thus, their contribution to the N economy of the soil can be quite substantial.

Moreover, the process is believed to consume less energy than nitrogen fixation through the mineral process (Dubey, 2006). For these reasons, inoculation with strains of rhizobia has become an important agronomic practice to augment N supply to legumes such as soybean and reduce the amount of inorganic N fertilizers required. In addition, legumes are presumed to rely less on external inorganic N sources (that is mineral fertilizers) than non-leguminous crops, such as cereals. It is pertinent to note that other kinds of bacteria, for example, the free-living N_2-fixing bacteria (*Azotobacter* and *Azospirillum*) and phosphate-solubilizing bacteria (*Bacillus* and *Pseudomonas*) are equally important in cropping systems. Hence, there is a growing interest in inoculating seeds of cereal crops with *Azospirillum* sp. because of the perceived symbiosis between the bacterium and the roots of grasses (Dewana et al., 1979; Dubey, 2006). It has been reported that *Azospirillum* sp., *Herbaspirillum* sp., *Azotobacter* sp. and *Acetobacter* sp. occur extensively in economically significant crop plants (e.g., maize, wheat, rice, sorghum, and sugar cane) that may also have some agronomic benefits (Reinhold and Hurek, 1988; Sundaram et al., 1988; Döbereiner, 1997). In addition, *Bacillus* sp. promotes plant growth through enhanced uptake of nutrient elements such as N, P, potassium (K), and iron (Fe) (Verma et al., 2010).

Also, the colonization of plant roots by *Trichoderma* sp. often provides some benefits, such as enhanced uptake and use of nutrients as a result of improved root growth and development, and resistance to abiotic stresses. It is known that strains of *Fusarium* sp. have the capacity to colonize plant roots (Katan, 1971; Gordon et al., 1989) and can even contribute to plant health through the suppression or prevention of disease (Larkin et al., 1993); this is an integral aspect of soil conditioning. Although there is evidence that rhizobial and mycorrhizal inoculants can improve the yields of certain crops (Giller, 2001), there is a lack of scientific evidence of the impact of most biological and chemical products currently being offered to farmers to improve crop productivity in sub-Saharan Africa. This study was therefore, conducted to:

1. Assess the effects of some commercially produced bio-inoculants and indigenous soil microbial isolates on the growth and DMY of maize and soybean,
2. Determine the effectiveness of commercial formulations containing *Bacillus* spp. and *Trichoderma* spp. as bio-control agents, and
3. Determine the effectiveness of free-living N-fixing microorganisms in increasing the uptake of N in maize.

MATERIALS AND METHODS

Indigenous soil microbial isolates

Bacillus spp. was isolated from the soil by the pour plate method described by Harrigan and McCance (1966). The microorganism was cultured by inoculating a loopful of the isolate into about 60 ml of yeast extract mannitol broth (YMB) contained in a 125-ml conical flask. The flask was placed on a reciprocal shaker (100 rpm) in a controlled-environment incubator at 30°C for 5 days. *Azospirillum* spp. was isolated from roots of upland rice using selective N-free bromothymol (NFB) medium (Döbereiner et al., 1976).

Culturing was done by introducing a loopful of the bacteria into conical flasks containing synthetic malate (20 ml) that were incubated for 3 days at 30°C on a reciprocal shaker (100 rpm). The conditions for growth were selected to discourage the aggregation of cells (Reinhold et al., 1985). The concentration of both inocula was ascertained by measuring their absorbance at 600 nm with a spectrophotometer. The *Bacillus* spp. inoculum had an absorbance of 0.163 which signified a concentration of 6.1×10^{11} CFU ml^{-1} and that of the *Azospirillum* spp. inoculum was 0.360 which indicated a concentration of 7.7×10^{9} CFU ml^{-1}. Spores of *Trichoderma viride* and *Fusarium oxysporum* were quantified with a haemocytometer. A standard spore concentration of each organism was prepared by adding 50 ml of water and a drop of Tween 80 to 7-day-old plate cultures and suspensions passed through sterile muslin cloth. Serial dilution was carried out and spore concentrations of 1.57×10^{7} CFU ml^{-1} were used for both *T. viride* and *F. oxysporum*.

Soil

Soils were collected at a depth of 0 to 15 cm from farmers' fields in Sudan savanna (SS), southern Guinea savanna (SGS), and derived savanna (DS) agroecological zones of Nigeria. The actual locations of the fields were Shanono in SS, Mokwa in SGS, and Ibadan in DS. The soils were air-dried, passed through a 4-mm mesh and thoroughly homogenized. Subsamples of the soils were taken for laboratory analyses (IITA, 1982) of selected physical and chemical properties (Table 1). The soils were weighed into PVC tubes (diameter = 15.24 cm and height = 43 cm), closed at the bottom with double nylon 1 mm mesh for the greenhouse trial. These tubes are referred to as pots hereafter. Because of differences in bulk density, soil mass pot^{-1} was 9.5 kg for Shanono and Ibadan soils and 10.5 kg for Mokwa soil.

Table 2. List of the commercial products and indigenous soil microbial isolates evaluated

Product/inoculum	Source	Formulation	Probable microorganisms
Mazospirflo-2	Soygro Ltd, South-Africa	Liquid	*Azospirillum brasilense*, Strain AL (1×10^9 CFU ml^{-1}).
Eco-T	Plant Health Product (Pty) Ltd. South Africa	Powder	*Trichoderma harzianum*, Strain Rifai KRL AG2 (2×10^9 CFU ml^{-1})
PHC Biopak	Plant Health Product (Pty) Ltd. South Africa	Peat	Various *Bacillus* spp. and *Paenibacillus azotofixans* (2×10^{10} CFU kg^{-1})
Azospirillum	Rice root	Liquid	Various *Azospirillum* spp..
Bacillus	Soil	Liquid	Various *Bacillus* spp.
Trichoderma viride	Soil	Liquid	*Trichoderma viride*
Fusarium oxysporum.	Cowpea	Liquid	*Fusarium oxysporum*

Experimental setup

The commercial products and the microbial isolates (Table 2) were evaluated in a greenhouse experiment at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, using as test crops one variety each of maize (*Zea mays*, cv. TZE-Y POPDT STRC 4) and soybean (*Glycine max*, cv. TGx 1448-2E). These crops are important components of cropping systems in the region. Soybean is often intercropped or grown in rotation with maize or other cereal crops and is reported to improve the nutritional status and welfare of resource-poor farmers (Sanginga et al., 1999). The selected varieties are high yielding and widely used in Nigeria. The experiment was conducted in two parts. One part involved products or isolates that contained free-living N-fixing microorganisms which were applied to maize in Ibadan, Mokwa, and Shanono soils. The other consisted of products or isolates that contained bio-control agents which were evaluated on soybean in sterilized and non-sterilized Shanono soil. The treatments included a reference termed 'mineral N' into which all nutrient elements were added at optimal rates to eliminate every possible nutrient limitation, and a control that received only macro-nutrients (N, P, K, S, and calcium) at suboptimal rates (that is 20% of the optimal rate). Suboptimal rates were chosen to create suitable conditions for the microbial inoculants to express their full potential or for the plants to maximize the benefits of the biofertilizers. The other treatments (that is with the addition of commercial products or microbial isolates and their combinations) also received only macro-nutrients at suboptimal rates). Nutrient calculations were based on tissue concentrations at the optimal level and the expected DMY $plant^{-1}$. Nutrient additions at optimal rates contained per pot: 4.33 g N; 1.8 g K; 0.92 g S; 1.98 g Ca; 2.0 g Mg; 0.026 g Mn; 0.006 g Zn; 0.003 g Cu; 0.001 g Co; 0.016 g B; and 0.001 g Mo. Because of differences among the soils in bulk density and P requirement, different amounts of P were added; Ibadan and Shanono soils received 3.58 g pot^{-1}; Mokwa soil received 3.96 g pot^{-1}. The soils in the pots were thoroughly mixed with the appropriate nutrient solutions and the moisture content was adjusted to about 70 % of field capacity with de-ionized water. The pots were allowed to stand overnight before planting was done.

Ten seeds of soybean and five of maize were planted in appropriate pots; seedlings were thinned to one after emergence. Prior to planting, all seeds were washed with 95% ethanol and 3% hydrogen peroxide to reduce microbial load. The treatments were replicated four times and the pots were arranged on greenhouse benches following a completely randomized design. In addition, the pots were re-arranged occasionally to eliminate location effects. The mode and time of application of the commercial products (Eco-T, Mazospiriflo-2, and PHC Biopak) and indigenous soil microbial isolates (*Azospirillum* spp., *Bacillus* spp., *Trichoderma* spp., and *Fusarium* spp.) are shown in Table 3. They were applied separately and in combination involving the same quantity of each product as in the single applications. The combinations were *Azospirillum* + Mazospiriflo-2, *Fusarium* spp. + Eco-T, *Fusarium* spp. + *Trichoderma* spp., *Fusarium* spp. + *Bacillus* spp., and *Fusarium* spp. + PHC Biopak. During plant growth, the moisture content was restored daily by the addition of appropriate amounts of de-ionized water to the pots. The plant height was measured with a meter rule at weekly intervals beginning at 3 weeks after planting (WAP). The average temperatures in the greenhouse during plant growth were 22°C minimum and 44°C maximum.

The plants were harvested at 8 WAP by cutting the shoots at 0.5 cm above the soil surface with thoroughly cleaned secateurs. Subsequently, the roots were separated by washing away the soil over a 4-mm sieve. For soybean, nodules were detached from the roots, counted, and weighed to obtain fresh weight and oven-dried for the dry weight determination. The roots and shoots were initially air-dried in clean paper bags and later oven-dried at 65°C for 72 h for dry matter determination. The shoots were ground and subsamples were analyzed for N and P concentrations (IITA, 1982). Estimation of BNF by soybean was done by the ureide method, as described by Herridge et al. (1990), using hot-water extracts of stems and petioles. Because nodules were not formed by soybean grown in the sterilized soil, it was assumed that N was not acquired through BNF in that soil. Therefore, the estimated BNF values for soybean in the sterilized soil were subtracted from those measured in the unsterilized soil where soybean formed nodules and, expectedly, acquired some amounts of N through BNF to derive the percentage of total N in the soybean attributable to BNF.

Statistical analysis

Statistical analysis of the data was performed with the Mixed Model procedure of the Statistical Analysis System version 9.2 (SAS, 2009) to assess treatment effects and their interactions. Replication was set as the random effect. Treatment differences were assessed by separating computed LSMEANS by pair-wise comparison using the PDIFF option of SAS at 5% level of significance. Data on the number of nodules formed by soybean were

Table 3. Mode of application of the commercial products and indigenous soil isolates

Product	Mode of application	Rate and time of application
Mazospirflo2	Seed inoculation, 200 ml per 25 kg of seed.	0.167ml per maize seed at planting
Eco-T	Seed inoculation, 1 g per kg seed	0.02 g per soybean seed at planting
PHC Biopak	Soil inoculation, 2-teaspoonsful per gallon of water	375 per pot (split applied: ⅓ at planting, 2 and 4 WAP)
Azospirillum	Soil inoculation	5 ml pot^{-1} (CFU ml^{-1} = 7.7 × 10^9); at planting
Bacillus	Soil inoculation	10 ml pot^{-1} (CFU ml^{-1} = 6.1 × 10^{10}); at planting
Fusarium oxsporum	Soil inoculation	10 ml pot^{-1} (CFU ml^{-1} = 1.57 × 10^7); at planting
Trichoderma viride	Soil inoculation	10 ml pot^{-1} (CFU ml^{-1} = 1.57 × 10^7); at planting

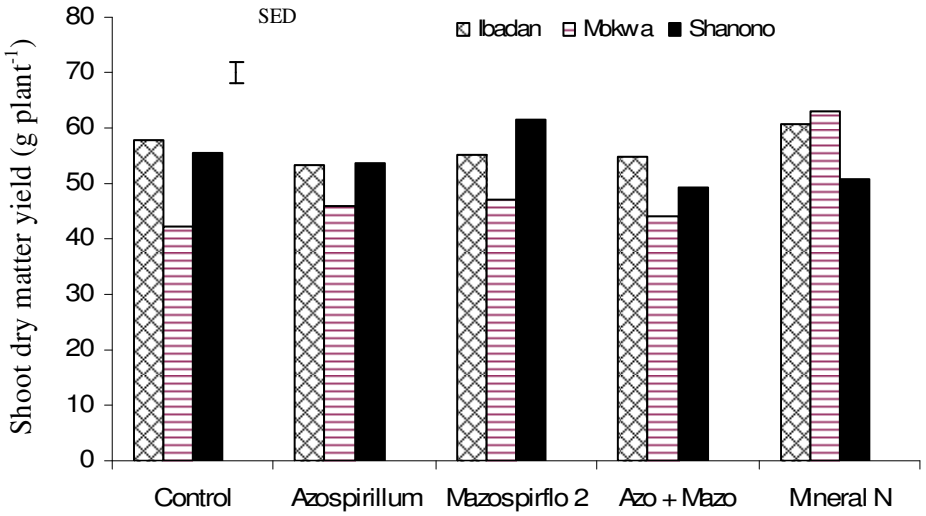

Figure 1. The effect of the microbial inoculants and mineral N fertilizer on the shoot dry matter production of maize in Ibadan, Mokwa and Shanono soils under greenhouse conditions. 'Azo+Mazo' is a combination of *Azospirillum* and Mazospirflo 2. Bar represents standard error of difference (SED).

transformed using Log (number + 1) before analysis.

RESULTS AND DISCUSSION

Maize dry matter yield, shoot N and P accumulation

Generally, shoot DMY was relatively large (42 to 63 g $plant^{-1}$) and differed significantly among the soils. However, inoculation with either *Azospirillum* or Mazospiriflo-2 did not improve the shoot DMY significantly (P > 0.05) in any of the soils when compared with the control (Figure 1). Although *Azospirillum* has been shown to colonize the roots of many species and also contribute positively to the growth and yield of many of those plants (Bashan and Levanony, 1990; Bashan, 1993; Okon and Labandera-Gonzales, 1994; Bashan and Holguin, 1995), neither the indigenous soil isolate nor the commercially produced inoculum was effective in the present study. The shoot to root ratio was similar in all treatments. Accumulation of N in the shoot biomass was dependent on the soil, as a significant interaction between treatment and soil was observed (Figure 2). A comparison of the control with other treatments within a soil showed that the application of Mazospiriflo-2 enhanced shoot N accumulation in Shanono soil (P = 0.006); in Mokwa soil, a significant (P = 0.0007) improvement in shoot N accumulation was recorded in the 'mineral N' treatment only. The increase in N accumulation in the shoots of maize grown in Shanono soil inoculated with Mazospiriflo-2 may be related to the inherent soil P content and, perhaps, to its improved availability due to bacterial action which in turn enhanced N uptake. Shanono soil had a larger amount (14 mg kg^{-1}) of plant-available P determined as Melhlich-P than Mokwa and Ibadan soils.

Although Mazospiriflo-2 is believed to consist of strains of *Azospirillum brasilense* at a concentration of about 1×10^9 CFU ml^{-1}, it is not clear whether other kinds of bacterial species were not present in the product as the purity was not tested. It was also evident from the data presented that inoculation with the indigenous soil isolate,

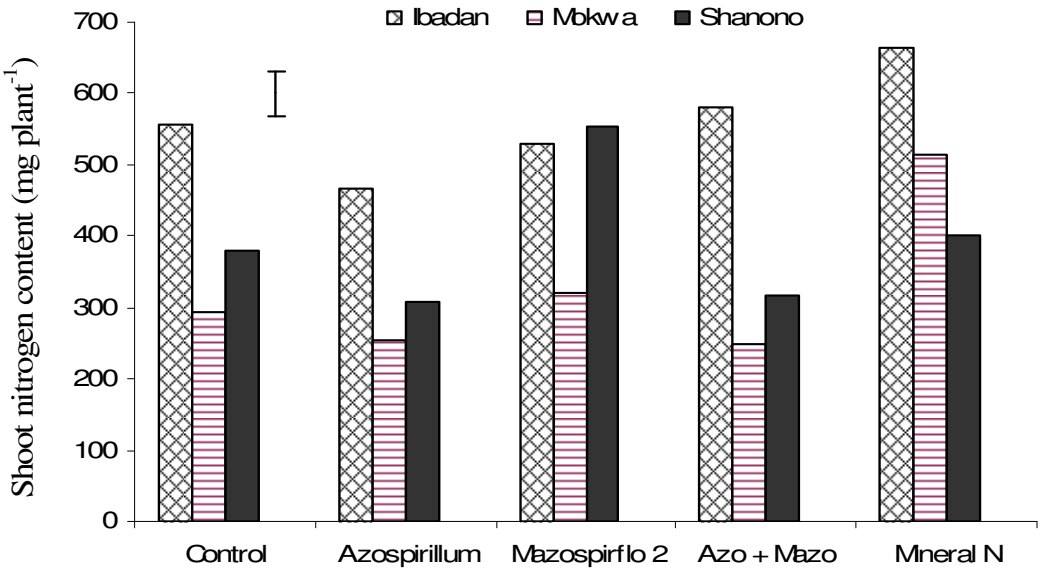

Figure 2. The effect of the microbial inoculants and mineral N fertilizer on nitrogen concentration in the shoots of maize grown in Ibadan, Mokwa, and Shanono soils under greenhouse conditions. 'Azo+Mazo' is a combination of *Azospirillum* and Mazospirflo 2. Bar represents standard error of difference (SED).

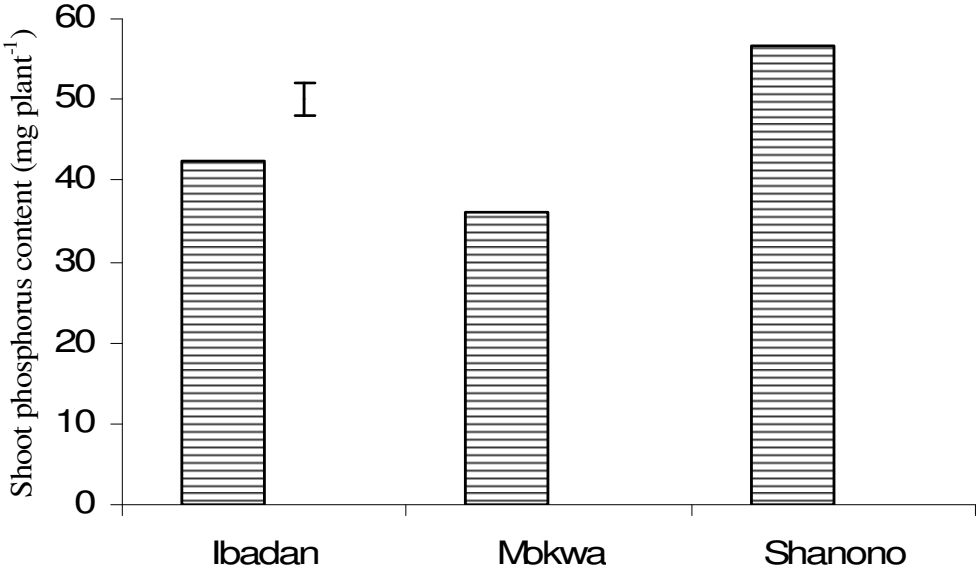

Figure 3. Phosphorus content of maize shoots (averaged across soils) as affected by microbial inoculants and mineral N fertilizer applied to Ibadan, Mokwa, and Shanono soils under greenhouse conditions. Bar represents standard error of difference (SED).

Azospirillum spp. (a free-living N-fixing microorganism) did not enhance N uptake in maize, even though reports abound in literature of the beneficial effects of rhizobacteria on the growth of many plant species. For example, Dobbelaere et al. (2001, 2002) reported early development and better growth of spring wheat due to inoculation with *A. brasilense*. In addition, inoculation had no significant effect on shoot P accumulation ($P > 0.05$). However, the soil effect was significant and, on average, more P was measured in the shoots of maize grown in Shanono soil which had the highest amount of plant-available P than in those grown in Mokwa and Ibadan soils (Figure 3). Based on the significance level of F-test values for the various parameters related to maize growth (Table 4), the addition of the microbial products did not improve maize growth significantly.

Table 4. The effect of the microbial products and mineral N fertilizer, relative to the control (averaged across soils), on growth parameters in maize grown under greenhouse conditions.

Product	Shoot DMY	Root DMY	Shoot N	Shoot P	Shoot/Root ratio
Azospirillum	ns	(**)	ns	ns	(*)
Mazospirflo 2	ns	ns	ns	ns	ns
Azospirillum + Mazospirflo 2	ns	ns	ns	ns	ns
Mineral N	(**)	(*)	(**)	ns	ns

ns = Not significant at P < 0.05; (*) = positive effect at P < 0.05; (**) = positive effect at P < 0.01.

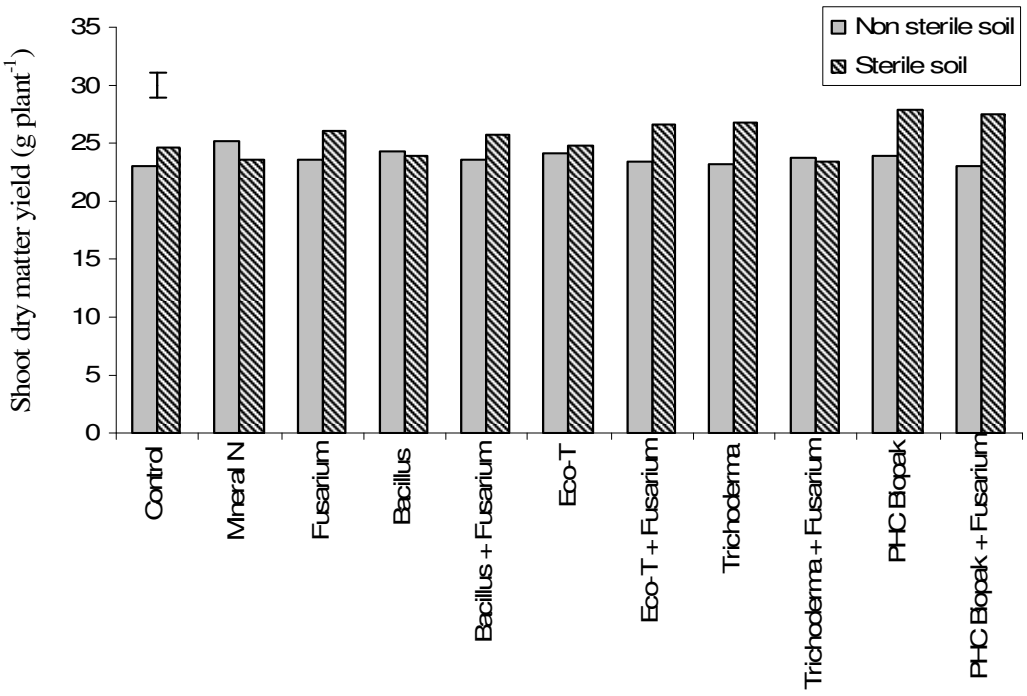

Figure 4. Shoot dry matter yield of soybean as affected by microbial inoculants and mineral N fertilizer applied to sterile and non-sterile soils under greenhouse conditions. Bar represents standard error of difference (SED).

Soybean dry matter yield, shoot N and P accumulation

The effect of the microbial inoculants and products on shoot DMY of soybean was not significant (P > 0.05). In essence, compared with the control, shoot DMY was not improved by the inoculation or addition of the microbial products (Figure 4). However, shoot DMY was slightly higher in the sterilized soil than in non-sterilized soil. Root DMY followed the same pattern as shoot DMY. The accumulation of N and P in the shoot biomass was not enhanced by the application of the microbial products but the amounts accumulated in sterilized and non-sterilized soils were significantly different (P < 0.001); larger amounts of N and P were taken up when the soil was sterilized (Table 5).

Generally, the growth of soybean was slightly better (P = 0.0043 for shoot DMY and 0.0002 for height) when the soil was sterilized. Inoculation with the microbial isolates and products significantly increased the heights of soybean plants in the sterilized soil (P < 0.05) compared with the control within that soil but no significant effect was observed when the soil was not sterilized. Sterilizing the soil may have eliminated or reduced possible competition between the plants and diverse microorganisms in soil for nutrients, particularly N, which enhanced the growth of the plants. Studies by Mu-qing et al. (2008) also showed that plants produce more biomass in sterile soil than in non-sterile soil. In general, the effects of the microbial inoculants on soybean growth were similar and no disease incidence was observed, even in treatments that received *F. oxysporum*. This is not strange because the ability of *Fusarium* sp. to protect plants from disease or be the basis of disease

Table 5. Mean values of selected growth parameters in soybean as affected by the microbial inoculants and mineral N fertilizer in sterile and non-sterile soils under greenhouse conditions.

Soil condition	Shoot dry matter (g plant^{-1})	Root dry matter (g plant$^{-1)}$	Shoot N content (mg plant^{-1})	Shoot P content (mg plant^{-1})	Pod dry matter (g plant^{-1})	Nodule dry matter (g plant^{-1})
Non sterile	23.7	7.1	443.2	36.2	1.49	0.6
Sterile	25.5	6.3	580.5	44.4	1.24	0.0
SED	0.61	0.24	32.68	2.55	0.146	0.026

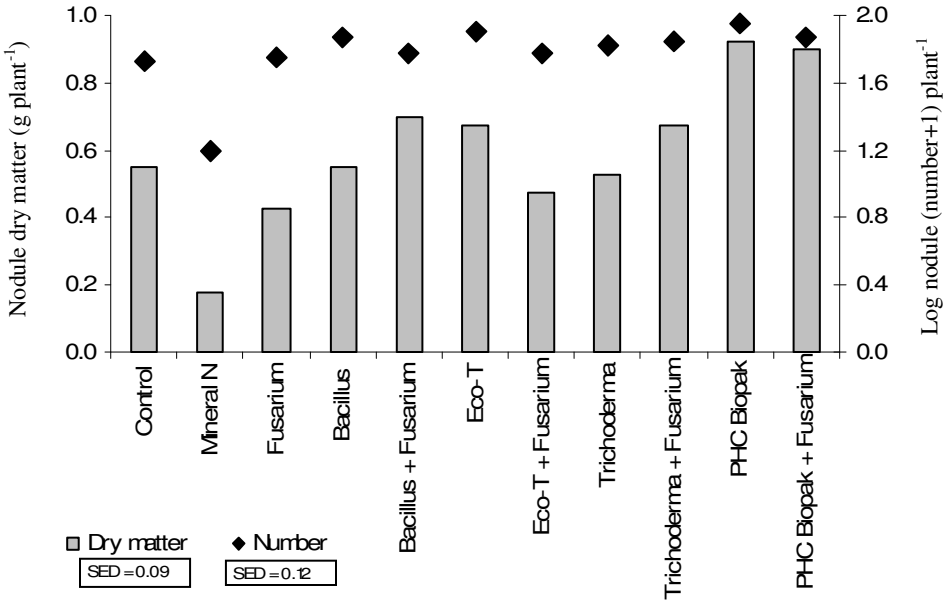

Figure 5. The number and dry weight of nodules formed by soybean under greenhouse conditions as affected by the application of microbial inoculants and mineral N fertilizer. Bar represents standard error of difference (SED).

prevention has been stressed (Larkin et al., 1993).

Nodule formation and N fixation by soybean

The number and dry weight of nodules formed by soybean are shown in Figure 5. The addition of PHC Biopak significantly improved the nodule dry weight compared with the control. Although the addition of most of the microbial products resulted in the formation of a similar number of nodules, those formed with the addition of PHC Biopak were larger than those formed by plants inoculated with *Fusarium* or Eco-T. The relatively large nodules formed in the PHC Biopak treatment could be attributed to a positive interaction between native rhizobial strains and the microorganisms that were in the product. This assertion is strengthened by the observation of Camacho et al. (2001) that some *Bacillus* spp. can enhance nodulation on bean plants when combined with a certain strain of symbiotic bacteria (*R. tropici*) since information provided by the producers indicated that PHC Biopak contains various *Bacillus* spp. and *Paenibacillus azotofixans* at 4.5 × 10^{10} CFU Lb^{-1} (equivalent to about 2 × 10^{10} CFU kg^{-1}).

Soybean grown in sterilized soil did not form nodules (Table 5). This is not strange since the sterilization process eliminated all indigenous microorganisms and the soil was not inoculated with rhizobium afterward. The proportion of N acquired by soybean through BNF was not significantly different among the treatments ($P > 0.05$). However, an average of about 38% of the total N content was estimated as biologically fixed nitrogen and when considered on absolute terms, the percentage of N fixed following inoculation with PHC Biopak was higher than with the other inoculants. The results obtained in this study indicate that inoculation with PHC Biopak has the potential to enhance nodule formation by soybean and probably N_2 fixation.

Single inoculation versus combined inoculation

In maize, a mixture of *Azospirillum* and Mazospiriflo-2 was applied, but the combination did not influence the

shoot DMY significantly except in Shanono soil where it resulted in a significantly (P = 0.0006) lower shoot DMY than the sole application of Mazospiriflo-2. The combined inocula also reduced N accumulation in the shoot biomass of maize grown in Shonono soil compared with the single inoculation with Mazospiriflo-2. This observation of antagonism among the bacterial species suggests that a combined application may not be ideal for maize. In soybean, a comparison of a single inoculation with the combined inoculation of the bio-inoculants with respect to shoot DMY and nutrient accumulation also revealed that both approaches yielded similar results. A common expectation is a better result from a combined application of different root-colonizing bio-control agents, such as *Trichoderma* and *Fusarium*, than from a single application of any one agent (Whipps, 2001). This expectation was not fulfilled in the present study, even though *Trichoderma* has the potential to influence crop yields positively, particularly under suboptimal conditions (Harman, 2000).

However, it is important to state that the outcome of combined inoculation depends on the strains of the rhizobacteria and the nature of the host plant. For example, Camacho et al. (2001) reported an increased nodulation on *Phaseolus vulgaris* (common bean) when *Bacillus* sp. strain CECT 450 was combined with *R. tropici* strain CIAT 899 under controlled and field conditions but this combination had a negative effect on root growth in soybean. They also observed that a single inoculation of CECT 450 produced no significant effect on bean plants. Another good example is the report of Rokhzadi et al. (2008) that a combined inoculation of *Azospirillum* spp., *Azotobacter chrococcum 5*, *Mezorhizobium ciceri* SWR17, and *Pseudomonas fluorescens* P21 strains improved biomass and grain yields of chickpea under field conditions better than single or combined inoculation without *P. fluorescens* P21. The authors attributed the increase in yield to the cumulative effects of the rhizobacteria, particularly on the supply of nutrients to the crop and the production of growth promoting substances of which *P. fluorescens* P21 played a key role.

In conclusion, the beneficial effects of the bio-inoculants in terms of an increase in DMY were not significantly evident. However, Mazospiriflo-2 seems to have the potential to enhance N uptake in maize in moderately fertile soils considering the observed increase in shoot N content when Shanono soil was used (Figure 2). Although an improvement in the growth and yield of soybean due to the bio-inoculants was not apparent, inoculation with PHC Biopak appeared to enhance nodule mass which may support N-fixation, and thus contribute to the N-economy of the soil. Even though the bio-inoculants failed to improve the shoot DMY of maize under greenhouse conditions, evaluations are imperative under field conditions where environmental effects cannot be avoided.

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