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Belowground Influence of *Rhizobium* Inoculant and Water Hyacinth Composts on Yellow Bean Infested by *Aphis fabae* and *Colletotrichum lindemuthianum* under Field Conditions

Victoria Naluyange[1], Dennis M. W. Ochieno[1], Philip Wandahwa[2], Martins Odendo[3], John M. Maingi[4], Alice Amoding[5], Omwoyo Ombori[4], Dative Mukaminega[6] & John Muoma[1]

[1] Department of Biological Sciences, Masinde Muliro University of Science and Technology (MMUST), Kakamega, Kenya

[2] School of Agriculture, Veterinary Science and Technology, Masinde Muliro University of Science and Technology (MMUST), Kakamega, Kenya

[3] Socio-Economics and Statistics Division, Kenya Agricultural and Livestock Research Organization (KALRO), Kakamega, Kenya

[4] Department of Plant and Microbial Sciences, Kenyatta University, Nairobi, Kenya

[5] Department of Soil Science, Makerere University, P. O. Box 7062, Kampala, Uganda

[6] Faculty of Applied Sciences, Kigali Institute of Science and Technology (KIST), P.O. Box 3900, Kigali, Rwanda

Correspondence: Victoria Naluyange, Department of Biological Sciences, Masinde Muliro University of Science and Technology (MMUST), P.O. Box 190-50100, Kakamega, Kenya. E-mail: vicluy@gmail.com

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Abstract

Rhizobium inoculant has been developed for bean production in Lake Victoria basin. Two types of compost have been developed, water hyacinth compost with cattle manure culture (H+CMC) or with effective microbes (H+EM). Influence of *Rhizobium* and composts on *Aphis fabae* and *Colletotrichum lindemuthianum* were investigated in the field. *Rhizobium* and hyacinth composts increased nodulation (×2 to 5); while *Aphis fabae* population increased (×2) on *Rhizobium*-inoculated plants with H+EM. Incidence of *C. lindemuthianum* was high in *Rhizobium*-inoculated plants. Plants that received diammonium phosphate (DAP) fertilizer had few nodules, reduced germination, slow growth and low yields. In conclusion, the water hyacinth composts contain beneficial microbes that promote root nodulation by *Rhizobium*, which is necessary for nitrogen fixation, while enhancing tolerance to aboveground infestations by *A. fabae* and *C. lindemuthianum*. We raise questions on our results to stimulate research, considering that bean breeding programs in Africa have mainly focused on microbial pathogens, and not insect pests.

Keywords: anthracnose, compost, manure, soil fertility, sustainability

1. Introduction

Common bean *Phaseolus vulgaris* is an important food security crop, and the major source of plant protein within the Lake Victoria basin in East Africa (David & Sperling, 1999). Beans complement the shortage of animal protein in East Africa, especially in the prevailing situation whereby fish production has been greatly impeded by the disastrous spread of water hyacinth in Lake Victoria (Ntiba et al., 2001; Hecky et al., 2010). The leguminous crop is also very important in agro-ecosystems, as it symbiotically fixes nitrogen through endophytic *Rhizobium* species (Bala et al., 2011; Devi et al., 2013). However, bean production has been declining to levels that are too low to meet the demand in East Africa (Mauyo et al., 2007). The main causes of declines in bean production are inferior germplasms, low soil fertility, pests and diseases (David & Sperling, 1999; Danielsen et al., 2013; Tittonell & Giller, 2013). Depletion of soil nutrients such as nitrogen and phosphorus has been a growing problem for bean production in East Africa (Kimani et al., 2007, 2008; Ayuke et al., 2011). Insect pests such as the black bean aphid *Aphis fabae* have been transmitting viral diseases (Beebe, 2012; Were et al., 2013), while fungal pathogens such as *Colletotrichum lindemuthianum* cause anthracnose disease of beans in East

Africa (Beebe, 2012; Kharinda, 2013). These factors have been complicated by the fact that local cultivars that are widely grown by smallholder farmers in East Africa have been succumbing to a complex of biotic and abiotic stresses (Otsyula et al., 2004; Ojiem et al., 2006; Kharinda, 2013).

There have been efforts to enhance sustainable crop production on farmlands in the Lake Victoria basin (Mireri et al., 2007; de Graaff et al., 2011), while conserving the lake for fish production (Lung'ayia et al., 2001; Nunan, 2013). Among the strategies, *Rhizobium* inoculants are being developed to enhance legume production by fixing nitrogen in the Lake Victoria basin (Bala et al., 2011). At the same time, nutrient-rich water hyacinth in the heavily eutrophied lake is being processed into compost, and transferred onto nutrient depleted farmlands for crop production (Naluyange et al., 2014). Removal of water hyacinth from Lake Victoria restores conditions that are favorable for fishing. Furthermore, water hyacinth compost contains phosphorus (Gunnarsson & Petersen, 2007; Naluyange et al., 2014), which is necessary for nodulation and nitrogen fixation in *Rhizobium*-inoculated bean seeds (Ssali & Keya, 1983), through processes such as enhancement of plant growth (Robson et al., 1981), improvement of shoot metabolism (Jakobsen, 1985) and specific roles in nodule initiation, growth and function (Israel, 1987). Such specific roles of phosphorus include ATP synthesis for nodule development and function (Ribet & Drevon, 1996), as well as for signal transduction and cell membrane biosynthesis (Graham & Vance, 2003). *Rhizobium*-inoculated beans are being promoted for improved yields and nitrogen fixation in the Lake Victoria region (Kihara et al., 2010; Thuita et al., 2012).

Rhizobium inoculants, when applied on legumes, have belowground effects such as enhanced root nodulation associated with better plant growth (Graham & Vance, 2000). Such beneficial processes of *Rhizobium* are affected by biotic factors like rhizosphere microbes (van Veen et al., 1997) as well as abiotic factors like soil fertility (Rotaru & Sinclair, 2009). Belowground colonization of roots by *Rhizobium* has been found to interact with aphids and other aboveground herbivores (Kempel et al., 2009; Katayama et al., 2010, 2011; Martinuz et al., 2012). For example, root colonization by *Rhizobium* has been found to promote plant resistance to insect pests (Thamer et al., 2011). However, *Rhizobium* colonization of roots has also been related to an increase in aphid and fungal incidences on leguminous shoots, which has been attributed to improved nutritive suitability of the host plant due to nitrogen fixation (Dean et al., 2009, 2014; Naluyange et al., 2014). Such effects of *Rhizobium* on legumes are modified by soil fertility amendments (El-Wakeil & El-Sebai, 2009; Dean et al., 2009). For instance, *Rhizobium* when applied using water hyacinth compost as an inoculant carrier improves the growth of faba bean (Mohamed & Abdel-Moniem, 2010).

In the Lake Victoria basin, combined application of water hyacinth compost and commercial *Rhizobium* inoculant had some positive effects on performance of the commercial Rosecoco bean cultivar, depending on the water hyacinth compost formulation (Naluyange et al., 2014). However, farmers in the Lake Victoria basin, especially in Western Kenya, mostly rely on local bean cultivars obtained from the market, including the yellow bean '*Mugasa*' (David & Sperling, 1999; Otsyula et al., 2004). Bean seeds from the local markets attain higher germination percentage than the certified commercial varieties (Otsyula et al., 2004). Furthermore, smallholder farmers in the Lake Victoria region rarely coat their seeds with fungicides that are always present in the commercial seeds. The absence of fungicides in the local bean seeds makes them ideal for *Rhizobium* inoculation, since such chemicals are potentially harmful to the inoculants (Graham & Vance, 2000; Stoddard et al., 2010). However, unlike commercial bean cultivars, the yellow bean is among local cultivars that are yet to be studied, especially in terms of *Rhizobium* nodulation and pest infestations under the influence of soil fertility amendments such as water hyacinth compost.

The objective of this study was to determine the influence of *Rhizobium* inoculant and water hyacinth composts on the performance of yellow bean in terms of growth and yields, and how these applications affect natural infestation of the plants by *A. fabae* and *C. lindemuthianum*. We hypothesize that water hyacinth composts and *Rhizobium* inoculant contain plant growth promoting microbes that improve belowground nutrient acquisition for yellow bean growth and yields, enabling the plants to tolerate aboveground infestations by *A. fabae* and *C. lindemuthianum*.

2. Materials and Methods

2.1 Experimental Design

The field experiment was conducted at the Masinde Muliro University of Science and Technology farm (N 00 17.104', E 034° 45.874'; altitude 1561m a.s.l.). Soils in this region have been classified as dystro-mollic Nitisols (FAO, 1974; Rota et al., 2006). Nutrient composition for the soil was; total phosphorus (18.9 ppm), total nitrogen (0.26 %), organic carbon (2.5 %), potassium (0.41 $cmol_c\ kg^{-1}$), sodium (0.1 $cmol_c\ kg^{-1}$), calcium (2.3 $cmol_c\ kg^{-1}$), magnesium (0.8 $cmol_c\ kg^{-1}$), zinc (1.9 ppm) and iron (0.37 ppm), with acidic pH of 4.2 (Naluyange et al., 2014).

The experiment was laid out in a randomized block design comprising 2 × 4 factorial treatments with *Rhizobium* inoculum factor having two levels (with or without inoculation) and fertility factor with four levels i.e. no fertilizer (Non), diammonium phosphate fertilizer-DAP (18-46-0), water hyacinth compost + cattle manure culture (H+CMC), and water hyacinth compost + effective microbes (H+EM). Each of the resulting 8 treatment combinations (plots) had 25 plants (n) in 3 blocks (i.e. N=600). Each plot was in form of a row containing the 25 plants spaced at 20 cm, with a distance of 40 cm between the plots, without border rows. The treatment rows were completely randomized to minimize non-experimental bias in sampling for natural infestations of aphids and anthracnose disease on bean plants. This experiment was conducted during the long rain season between 20th April to 30th July 2012, and then repeated between 30th May and 31st August 2012 (Figure 1).

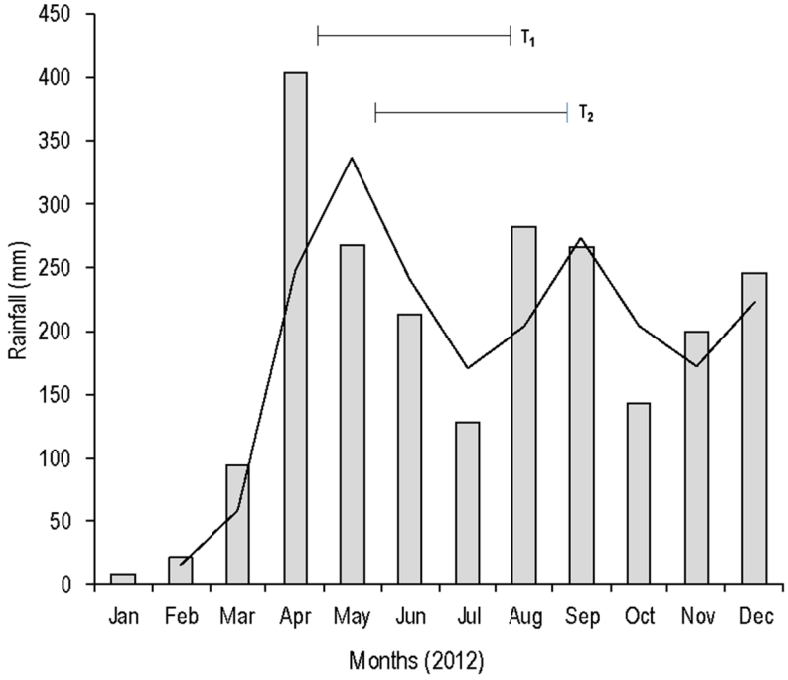

Figure 1. Rainfall in Kakamega county of Western Kenya in the year 2012 during the first field trial (T 1) between 20th April to 30th July 2012, and the second field trial (T 2) between 30th May and 31st August 2012. Total rainfall during the first trial was 1012 mm with a 3-month average of 253 mm. Total rainfall during the second trial was 622 mm with a 3-month average of 207 mm (Source: Naluyange 2013; Courtesy of the Kenya Agricultural and Livestock Research Organization (KALRO), Kakamega, Kenya)

2.2 Water Hyacinth Composts

Two formulations of compost made from water hyacinth + cattle manure culture (H+CMC) and water hyacinth + effective microbes (H+EM) were prepared using aboveground closed aerobic heap design (Naluyange et al., 2014). The H+CMC compost formulation was prepared by mixing chopped and dried water hyacinth material with a culture of decomposed cattle manure to supply saprophytic microbes. The H+CMC compost had a density of 58 g / 100 cm^3, with a nutrient concentration of total phosphorus (375 ppm), total nitrogen (1.1 %), organic carbon (13.4 %), potassium (21 $cmol_c\ kg^{-1}$), sodium (1.9 $cmol_c\ kg^{-1}$), calcium (22.3 $cmol_c\ kg^{-1}$), magnesium (12 $cmol_c\ kg^{-1}$), zinc (2 ppm) and iron (1.9 ppm), with alkaline pH of 8.1 (Naluyange et al., 2014).

The H+EM compost formulation was prepared by mixing dried and chopped water hyacinth material with Effective Microorganisms solution (EM™), containing photosynthetic bacteria (*Rhodopseudomonas palustris*), lactic acid bacteria (*Lactobacillus plantarum* and *L. casei*), yeast (*Saccharomyces cerevisae*), molasses and water (EM Technologies Ltd, Embu, Kenya). The H+EM compost had a density of 62 g / 100 cm^3, with the nutrient concentration of total phosphorus (270 ppm), total nitrogen (1 %), organic carbon (13.5 %), potassium (24.5 $cmol_c\ kg^{-1}$), sodium (1.7 $cmol_c\ kg^{-1}$), calcium (27.5 $cmol_c\ kg^{-1}$), magnesium (15.3 $cmol_c\ kg^{-1}$), zinc (4 ppm) and iron (1.7 ppm), with alkaline pH of 8.4 (Naluyange et al., 2014).

2.3 Seed Inoculation and Planting

Seeds of the local yellow bean cultivar '*Mugasa*' were purchased from the Kakamega town market in Western Kenya. These are among uncertified seeds that are widely grown by farmers (Otsyula et al., 2004). The bean seeds were inoculated with *Rhizobium* inoculant powder as per manufacturer's directions (BIOFIX®, MEA Ltd, Kenya). The seeds (250 g) were mixed in gum Arabic solution (0.5 gum Arabic/ 5 mL of sterile lukewarm water). The gum Arabic-coated seeds (250 g) were mixed with the *Rhizobium* inoculant powder (1 g). Controls were coated with gum Arabic solution only.

Planting holes of ~200 cm^3 volume (i.e. ~5 cm diameter and ~10 cm deep) were dug using a shovel. The water hyacinth composts were applied using containers of 150 mL volumes per hole (i.e. ~90 g) as per the respective treatments and mixed with soil. Therefore, each planting hole received approximately 0.03 g phosphorus and 0.99 g nitrogen for the H+CMC compost; or 0.02 g phosphorus and 0.90 g nitrogen in case of the H+EM compost. For the DAP treatment, one leveled teaspoon (4.7 g) was mixed with soil in the planting hole (Naluyange et al. 2014). DAP fertilizer contains nitrogen (18 %) and phosphorus pentoxide P_2O_5 (46 %), with phosphorus (P) constituting 20 % of the total mass. Hence, every planting hole in the DAP treatment received 0.94 g phosphorus and 0.85 g nitrogen. One bean seed was sown in every planting hole at a depth of ~2 cm.

2.4 Data Collection

Data was recorded as described by Naluyange et al. (2014). The emergence date of every seedling was recorded independently, and used to determine the duration for germination. The number of seedlings that germinated out of the total number of seeds that were planted was used to calculate the germination percentage within 20 days from the planting date. When the first trifoliate leaves were fully formed in ~80% of the seedlings, plant height (stem base to petiole), length of the middle leaf (base to apex) and its width (widest part) were recorded. The date when the first flower of every plant appeared was recorded and used to calculate the duration for flowering. Ten days from the onset of flowering, 5 bean plants were randomly selected from each treatment per block for the estimation of number of root nodules associated with *Rhizobium* colonization. The bean plants were dug out with their root system still holding rhizosphere soil and transported to the laboratory in plastic bags. Rhizosphere soil from each bean plant was gently removed onto a white paper. Detached root nodules in the soil and those still attached to the roots were carefully quantified using a tally counter. This method maximized the collection and counting of root nodules. At harvest, the pods from every plant were packed in separate paper packets and sun dried for a period of five days; the weight of bean seeds per plant was recorded.

Aphid infestations on bean plants were recorded at the vegetative and flowering stage of bean growth. Three screw-capped vessels each containing 10 mL of 70% ethanol were placed on every treatment row of 25 plants. Aphids from every 8 plants per row were collected into each container using a camel hair brush from leaves and stems. The collected aphids were identified under a dissection microscope (Model Z45E, Leica Inc., USA) at × 10 magnifications using the features described by Martin (1983) and Holman (1998), and their absolute counts recorded using a tally counter. These insects have already been identified as the black bean aphid *A. fabae* (Naluyange et al., 2014). At the vegetative stage, the bean plants were also scored for incidence of anthracnose disease (*C. lindemuthianum*) i.e. the proportion of plants having anthracnose symptoms, characterized by dark brown to black lesions on leaves (Hagedorn & Inglis, 1986; Buruchara et al., 2010).

2.5 Statistical Analysis

Statistical analyses were conducted using SAS 9.1 software (SAS Institute Inc.) at $p < 0.05$ confidence level. Proc Means was used in the generation of descriptive statistics such as means and standard errors for nodule counts, aphid population, plant growth (duration and size) and yields (pod counts and seed weight). These data were graphically checked for normality using Proc Univariate; while Proc Transreg was used to find appropriate Box-Cox power transformations for normalization of data. Data for aphid population were log-transformed, while untransformed data were used for nodule counts. Frequencies germination (%) and anthracnose disease incidence (%) were generated using Proc Freq. For plant growth and aphid population, Analyses of Variance (ANOVA) between treatment means for the fertility, *Rhizobium* and trial factors were done by Proc Mixed using the three blocks as random effects and the two trials (or plant growth stage for aphid data) as repeated measures. Means for plant growth and aphid population were separated using Ls-means when treatment effects were significant ($p < 0.05$). Anthracnose disease incidences and germination percentages were analyzed by Proc Genmod (χ^2 test; binomial) and percentages compared using Proc Multtest. Germination percentage data for the two seasons were combined because they were similar. Percentage increase and decrease in nodulation, aphid populations, plant growth and yields were calculated using the formula:-

$$\frac{Xi - Xc}{Xc} \times 100\ \%$$

Whereby *Xc* = mean for untreated controls (Non); and *Xi* = mean for other treatments.

3. Results and Discussion

In this study, it was expected that plants treated with water hyacinth composts and *Rhizobium* inoculant would exhibit improved nodulation, growth and yields, while expressing tolerance to infestations by *A. fabae* and *C. lindemuthianum*. The average number of root nodules in the untreated plants was seven (mean = 7), but significantly increased in plants grown using the water hyacinth composts without *Rhizobium*, with H+CMC attaining an extra 200 % (i.e. +14 nodules) and H+EM scoring extra 278 % (i.e. +19 nodules), when compared to the untreated controls (df = 7, $\chi^2 = 1138$, $p < 0.0001$) (Figure 2). Because these plants had not been inoculated,

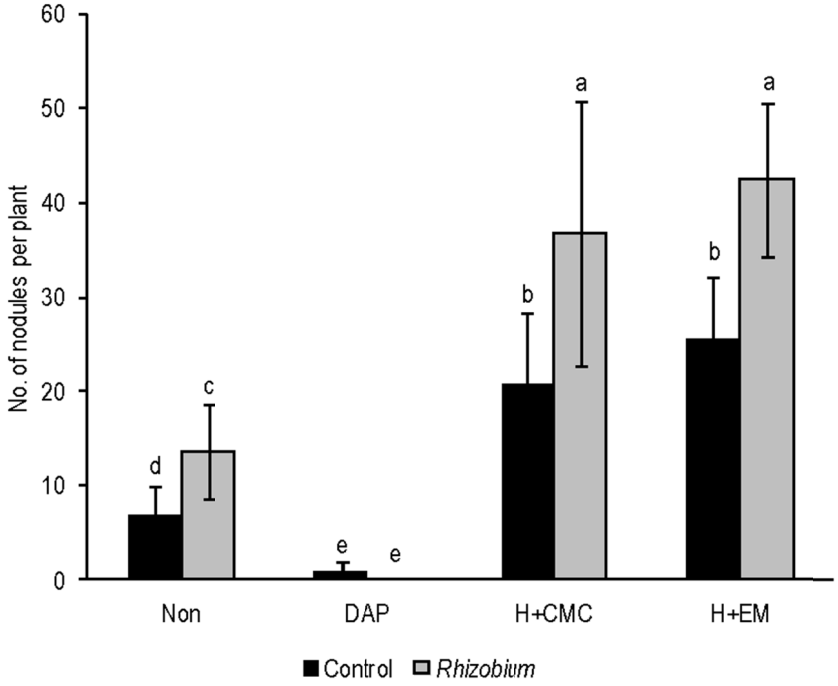

Figure 2. Number of root nodules in local yellow bean plants (var. *Mugasa*) as affected by commercial *Rhizobium* inoculant and soil fertility amendments; without fertilizer (Non), diammonium phosphate fertilizer (DAP), water hyacinth compost + cattle manure culture (H+CMC) and water hyacinth compost + effective microbes (H+EM). Bars with the same letter(s) are not significantly different (χ^2 test, $p > 0.05$)

the nodules are likely to have been formed by native strains of *Rhizobium* species (Dean et al., 2009; Naluyange et al., 2014). The *Rhizobium* inoculant, which significantly increased nodulation of plants grown without fertilizer by 100 % (i.e. +7 nodules), also performed better in combination with the water hyacinth composts, attaining high increases in nodulation at 440 % (i.e. +30 nodules) for H+CMC and 524 % (i.e. +36 nodules) in H+EM (Figure 2). These results on root nodulation are in line with the expectations that the water hyacinth composts and *Rhizobium* inoculant can promote root nodulation in the yellow bean, which indicates their compatibility (Naluyange et al., 2014). Microbes in the two water hyacinth composts may have enhanced root nodulation by *Rhizobium*, probably through phosphate solubilization (Argaw, 2012; Messele & Pant, 2012). Bean plants that received DAP had the lowest number of root nodules (Figure 2), despite the fertilizer being rich in phosphorus required for nodulation (Ssali & Keya, 1983; Graham & Vance, 2003). Reduction in nodule counts for DAP treatments ranged between -87 % to -100 % (i.e. zero nodules). The reduced nodulation has been linked to the chemical composition of inorganic fertilizers that limits the survival of Rhizobia (Peterson & Kremer, 1989). It is likely that the DAP inhibited *Rhizobium* nodulation, probably through acidification (Thawornchaisit & Polprasert, 2009). The soil was also already acidic with a pH of 4.2. Based on nutrient concentration, it is unlikely that nitrogen in DAP played a significant role in inhibiting root nodulation, because the levels of N

supplied to every plant in the three fertility treatments were similar i.e. DAP (0.85 g), H+CMC compost (0.99 g) and H+CMC compost (0.90 g). However, the ammonium form of nitrogen in DAP could have inhibited root nodulation and diazotrophic activities of *Rhizobium* inoculant (Huss-Danell et al., 1982; Mendoza et al., 1995). The form of nitrogen and phosphorus in the two water hyacinth composts was not established (Naluyange et al., 2014).

Composts and other soil fertility amendments can influence seed germination through properties such as water holding capacity (Celik et al., 2004) and chemical activities including phytotoxicity (Hartz et al., 1996; Kabir et al., 2010). Influence of the two water hyacinth composts (H+CMC and H+EM) on seed germination percentage and duration were not evident, as there was no difference with those grown without fertilizer ($p > 0.05$) (Figure 3). This was also the case in the commercial Rosecoco bean variety (Naluyange et al., 2014). Overall germination percentage for bean seeds in the first trial (74.8%) was significantly lower than in the second trial (81.4%) ($\chi^2 = 6.93$; $p = 0.0085$). However, there was no difference in germination percentage between the two trials when the DAP treatment was excluded in the statistical analysis ($df = 1$, $\chi^2 = 0.31$, $p = 0.58$). Variations in germination percentage between the two trials can be explained by the large difference in germination percentages of seeds grown with DAP, which were -87 % in the first trial and -56 % in the second trial. It is likely that the differences in climatic factors such as rainfall (Figure 1), may have affected the efficacy of DAP through soil moisture related processes (Olson & Dreier, 1956; Hoeft et al., 1975; Hartz et al., 1996; Salvagiotti et al., 2013). Rhizobia are known to improve seed germination by secretion of Nod-factors and phytohormones (Prithiviraj et al., 2003; Cassan et al., 2009). In the current study, there was no indication that the *Rhizobium* inoculant stimulated seed germination, but the inoculated seeds even exhibited further germination suppression when grown with DAP (Figure 3). This is an issue that requires investigation. Plants grown with DAP took longer period to germinate (Table 1), an effect that has been reported in other studies (Naluyange et al., 2014).

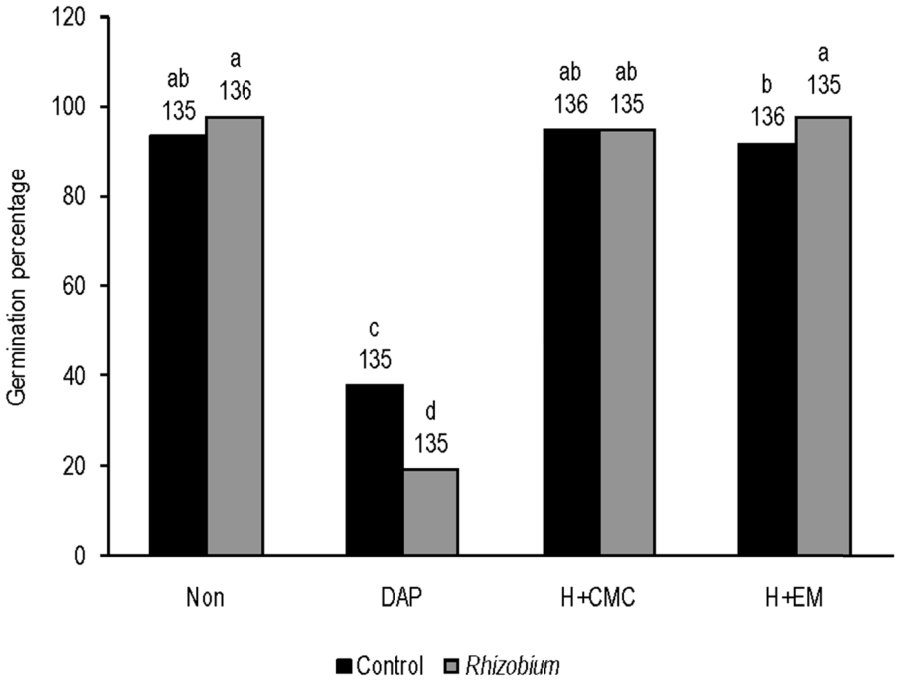

Figure 3. Germination percentage of local yellow bean seeds (var. *Mugasa*) as affected by commercial *Rhizobium* inoculant and soil fertility amendments; without fertilizer (Non), diammonium phosphate fertilizer (DAP), water hyacinth compost + cattle manure culture (H+CMC) and water hyacinth compost + effective microbes (H+EM). Numbers on top of bars represent sample sizes. Bars with the same letter(s) are not significantly different (χ^2 test, $p > 0.05$)

Beans grown with the two types of water hyacinth compost and those without fertilizer were not different in terms of plant size, days to flowering, as well as pod counts and seed weight per plot ($p > 0.05$) (Table 1). Furthermore, *Rhizobium*-related effects on the previously mentioned parameters were not detected (Table 1). In

the Rosecoco bean, plants grown with the two water hyacinth composts were large in size, and exhibited improved growth and yields in the second trial (Naluyange et al., 2014). Differences between the yellow bean and the Rosecoco in terms of response towards the two types of compost may be cultivar-related (Naluyange, 2013); although methodological differences such as statistical analysis approach need to be considered in this judgment. Yellow bean plants treated with DAP took the longest time to emerge and flower, while exhibiting reduced yields per unit area in terms of pod count (-64 %) and seed weight (-67 %) (Table 1). The low yields are a result of the low germination percentage in the DAP treatment (Figure 3). Despite reduction in germination percentage, individual mature plants from DAP treatments become large in size indicating that inhibitive effects of DAP are temporary (Naluyange et al., 2014). Such negative effects of DAP may be due to edaphic and climatic conditions specific to study locations (Ghizaw et al., 1999), because DAP has been reported to enhance bean yields in many other regions around the world (Zhang et al., 2008; Zafar et al., 2013). Bean plants in the first trial took shorter time to flower, were taller and produced higher number of pods with greater seed weight per unit area compared to the second trial (Table 1). The primary reason for this observation is the relatively more rainfall during the first trial (1012 mm) than in the second trial (622 mm) in which the relatively young bean plants were exposed to rain shortages in the month of July 2012 (Figure 1).

Table 1. Plant growth and yields of the local yellow bean '*Mugasa*' as influenced by *Rhizobium* inoculant, water hyacinth compost containing cattle manure culture (H+CMC) or effective microbes (H+EM) and diammonium phosphate (DAP) fertilizer under field conditions in Western Kenya

		Means of means						
		Emergence days	Flowering days	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	Pods (counts)	Yield (g)
Overall mean		6.90±0.24	40.32±0.46	8.37±0.32	5.44±0.18	5.16±0.12	125.29±12.58	125.2±13.06
First trial		6.87±0.14a	37.89±0.28 b	8.49±0.23a	5.76±0.12a	5.72±0.16 a	214.98±16.4 a	236.66±13.5 a
Second trial		6.81±0.10a	42.19±0.65 a	8.28±0.76a	5.22±0.47a	4.72±0.05 b	46.29±6.0 b	25.7±1.34 b
Fertilizer	Non	6.55±0.07 b	39.86±0.35 b	8.0±0.27a	5.17±0.16a	4.95±0.18 b	132±12.12 a	136.41±14.75 a
	DAP	8.66±0.37 a	43.36±0.99 a	7.39±1.11a	4.89±0.59a	4.72±0.28 b	47.17±26.12 b	45.63±28.68b
	H+CMC	6.26±0.17 b	38.88±0.35 b	9.28±0.21a	6.04±0.13a	5.72±0.17 a	175.17±14.56 a	167.94±15.82 a
	H+EM	6.15±0.13 b	39.18±0.25 b	8.8±0.25a	5.68±0.19a	5.24±0.06 ab	146.83±7.18 a	150.85±7.83a
Inoculum	Control	6.86±0.37a	39.98±0.49a	8.17±0.41a	5.34±0.24a	5.23±0.18a	113.75±15.19a	112.13±16.12a
	Rhizobium	6.95±0.31a	40.66±0.78a	8.56±0.49a	5.55±0.27a	5.09±0.16a	136.83±20.18a	138.28±20.56a
Source of variation	df	F values						
Trial	1,4	1.78	41.82**	0.07	1.25	30.03**	93.16***	836***
Fertilizer	3,16	28.58***	19.21***	1.52	2.06	3.93*	10.56***	33.57***
Inoculum	1,16	0.82	1.85	0.39	0.33	0.29	1.86	1.27
Fertilizer × Inoculum	3,16	0.82	1.68	0.17	0.04	0.12	0.39	0.27

Without fertilizer (Non), diammonium phosphate fertilizer (DAP), water hyacinth compost + cattle manure culture (H+CMC) and water hyacinth compost + effective microbes (H+EM). Asterisk indicates the significant effect, ***P ≤ .001, ** P ≤ 0.01, * P ≤ 0.05. Means with the same letter(s) are not significantly different; those with more than one letter are intermediate.

Populations of the black bean aphid *A. fabae* were generally low i.e. fewer than 1-2 aphids per plant in most treatments. *Aphis fabae* population was significantly higher in *Rhizobium* inoculated plants grown with hyacinth + effective microbes (H+EM) than the other treatments ($F_{7, 37}$; p = 0.0036) (Figure 4). This increase in *A. fabae* population by ~230 % may be linked activities of the *Rhizobium* inoculant, although microbes contained in the compost (H+EM) may have also contributed, as this effect was not evident in the other compost (H+CMC). It also implies that host-mediated effects of *Rhizobium* on aphids depend on the composition of microbial communities the legume symbiont interacts with in the rhizosphere (Whipps, 2001; Bais et al., 2006; Raaijmakers et al., 2009); as microbial communities that exist in cattle manure culture (Chachkhiani et al., 2004; Maeda et al., 2010), are unlikely to be similar to those in Effective Microbes® (Naluyange et al., 2014). Increase in aphid populations due to *Rhizobium* inoculation has also been reported in soybean and in the commercial Rosecoco bean (Dean et al., 2009; Naluyange et al., 2014). Since *Rhizobium* fixes nitrogen in legumes, then

increased *A. fabae* density may be linked to high organic nitrogen content that determines nutritive suitability of host plants (Mattson, 1980; Dean et al., 2009; Ballhorn et al., 2013). It is also possible that *Rhizobium* inoculated plants emit more attractive volatiles to *A. fabae*, a suggestion that requires investigation. However, in the Lima bean *Phaseolus lunatus*, the application of *Rhizobium* minimized olfactory attraction of the Mexican bean beetle (*Epilachna varivestis*) towards plants induced to produce attractive volatiles using jasmonic acid (Ballhorn et al., 2013). The *A. fabae* population did not vary between the vegetative and flowering stages of bean growth ($F_{1, 37}$; p = 0.33).

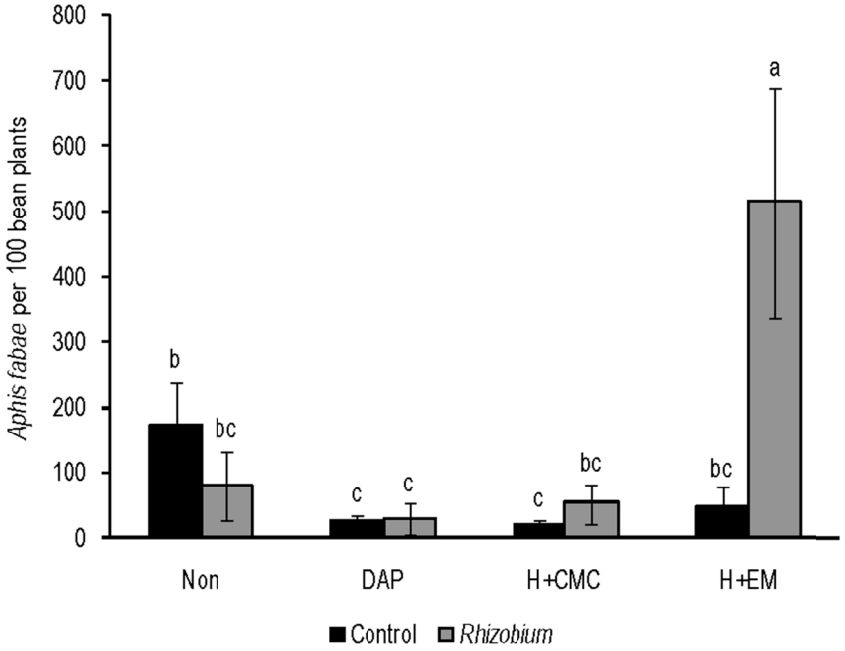

Figure 4. Population of *Aphis fabae* on local yellow bean plants (var. *Mugasa*) as affected by commercial *Rhizobium* inoculant and soil fertility amendments; without fertilizer (Non), diammonium phosphate fertilizer (DAP), water hyacinth compost + cattle manure culture (H+CMC) and water hyacinth compost + effective microbes (H+EM). Bars with the same letter(s) are not significantly different (F test, $p > 0.05$)

Incidence of anthracnose disease caused by *C. lindemuthianum* was significantly higher in *Rhizobium*-inoculated plants than in the non-inoculated plants, particularly in the first trial (Figure 5). It is likely that *C. lindemuthianum* infestations are dependent on *Rhizobium* activities, which can be supported by the similarity of trends in root nodule counts (Figure 2) and anthracnose incidence across fertility treatments (Figure 5). Just like *A. fabae*, this effect can also be linked to enhanced nitrogen content due to N_2 fixation. This is because *Colletotrichum* species that cause anthracnose disease and many other phytopathogens have high affinity for nitrogen in host plants (Nam et al., 2006; Tavernier et al., 2007; Lobato et al., 2009; Ochieno, 2010). On average, anthracnose incidence in the first trial (~62%) was higher than in the second trial (~7%). This is because the first trial had more rainfall (Figure 1), which favours *C. lindemuthianum* infestations on beans that are more prevalent under moist conditions (Kumar et al., 1999).

Apart from symbiotic nitrogen fixation, there could be other tripartite host-mediated interactions between *Rhizobium* species, the black bean aphid *A. fabae* and the anthracnose pathogen *C. lindemuthianum* that need to be established (Stout et al., 2006). First, feeding by aphids induces *Rhizobium* nodulation in leguminous roots (Heath & Lau, 2011). This is because the number of root nodules was higher in plants that had high *A. fabae* population, particularly in the H+EM compost; which is reverse of the assumption that *Rhizobium* is the one that causes increase in aphid infestation (Dean et al., 2009; Naluyange et al., 2014). This also contradicts scenarios in which *Rhizobium* reduces aphid population on crops through induced plant resistance (El-Wakeil & El-Sebai, 2009; Martinuz et al., 2012). *Rhizobium* may actually suppress plant immunity instead of boosting induced resistance (Mithofer, 2002; Luo & Lu, 2014). Secondly, feeding wounds inflicted by *A. fabae* stylets may have facilitated the entry of *Colletotrichum* hyphae into plant tissues. Since *Rhizobium* inoculated plants had more *A. fabae* and hence higher number of stylet wounds, then more *Colletotrichum* hyphae may have penetrated

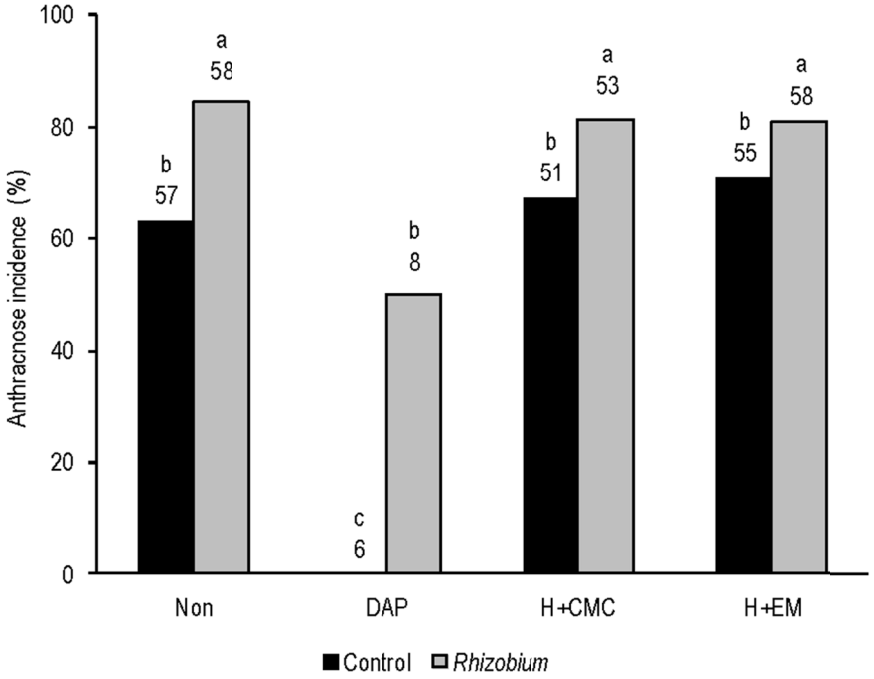

Figure 5. Anthracnose incidences in local yellow bean plants (var. *Mugasa*) as affected by commercial *Rhizobium* inoculant and soil fertility amendments; without fertilizer (Non), diammonium phosphate fertilizer (DAP), water hyacinth compost + cattle manure culture (H+CMC) and water hyacinth compost + effective microbes (H+EM). Numbers on top of bars represent sample sizes. Bars with the same letter(s) are not significantly different (χ^2 test, $p > 0.05$)

resulting in high anthracnose incidence. However, aphid stylet wounds are too small to permit the entry of fungal pathogens into plant tissues (Mitchell, 2004; Will & van Bell, 2006). Also, there are reports indicating that feeding by some aphid species inhibits plant tissue colonization by *Colletotrichum* species (Russo et al., 1997; Stout et al., 2006). Third, high number of aphids on *Rhizobium* inoculated plants may have secreted vast amounts of honeydew, which may have facilitated saprophytic colonization of bean surfaces by *Colletotrichum* hyphae prior to pathogenic penetration. However, this scenario is quite unlikely considering the low number of aphids (1-2) per plant. Fourth, both the aphid and the fungal pathogen may have compromised the immune system of the host plant to enhance their colonization (Stout et al., 2006). However, this interpretation may not fully stand, because plants have separate defense mechanisms against *Colletotrichum* and insect pests (Ajlan & Potter, 1991). Finally, plant infection by *Colletotrichum* may have influenced the production of aphid-attracting volatile organic compounds, because some endophytic fungi influence the production of insect attractive compounds (Cardoza et al., 2003; Jallow et al., 2008). However, this cannot be generalized, because *Colletotrichum* exhibits both biotrophic and necrotrophic infection phases (Bhadauria et al., 2011), which are associated with increase or decrease in *A. fabae* infestations on beans, respectively (Al-Naemi & Hatcher, 2013). Relationships between *Rhizobium*, *Colletotrichum* and aphids require further investigation.

Despite the high infestations by *A. fabae* and *C. lindemuthianum* in *Rhizobium*-inoculated plants grown with water hyacinth compost (H+EM), the growth and yields were not negatively affected, while the desirable root nodulation was enhanced. This indicates that the plants were tolerant to the insect pest *A. fabae* and the fungal pathogen *C. lindemuthianum* (Naluyange et al., 2014); although there are possibilities that plant growth promoting benefits of *Rhizobium* were cancelled by costs towards plant defensive mechanisms (Thaler et al., 1999; Heil et al., 2000).

In conclusion, the water hyacinth composts contain beneficial microbes that promote root nodulation by *Rhizobium*, which is necessary for nitrogen fixation, while enhancing tolerance to aboveground infestations by *A. fabae* and *C. lindemuthianum*. We raise questions on our results to stimulate research, considering that bean breeding programs in Africa have mainly focused on microbial pathogens, and not insect pests.

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