



Effects of NPK10-20-10 Chemical Fertilizer and Arbuscular Mycorrhizae on the Response of Common Bean (*Phaseolus vulgaris* L.) in an Acidic Soil of Lubumbashi Region

Bibich Kirika Ansey¹ · Audry Tshibangu Kazadi¹ · Jonas Lwalaba wa Lwalaba² · Mick Assani Bin Lukangila³ · Mylor Ngoy Shutcha⁴ · Geert Baert⁵ · Geert Haesaert⁶ · Robert-Prince Mukobo Mundende¹

Received: 22 November 2022 / Accepted: 21 March 2023 / Published online: 4 May 2023
© The Author(s), under exclusive licence to Springer-Verlag GmbH Deutschland, part of Springer Nature 2023

Abstract

Most soils in the Lubumbashi region are acidic (pH 4 to 6) and poor in available phosphorus. The production of crops such as common beans requires large amounts of chemical fertilizers, generally up to 200 kg ha⁻¹ of NPK10-20-10 compound fertilizer, recommended for good production, but which are not affordable for most of smallholder farmers. More over the pH range is not optimal for fertilizer efficiency. Under this circumstances fertilizers use efficiency can be made effective by arbuscular mycorrhizae (AMF) even when applied at low doses. In this study, four types of AMF inoculum (*Acaulospora*, *Gigaspora*, *Glomus* and *Acaulospora-Gigaspora-Glomus*) and an uninoculated control were selected and combined with two levels of NPK10-20-10 application, by bringing a quantity of products of 100 kg ha⁻¹, 200 kg ha⁻¹ and unfertilized plot as control. Application of NPK 200 kg ha⁻¹ reduced colonization frequency and AMF spore density with 28.5 to 41.8%, respectively. Plants that received 200 kg NPK ha⁻¹ associated to *Acaulospora-Gigaspora-Glomus* were less sensitive to bean fly than others. Application of 200 kg NPK ha⁻¹ as well as inoculation with *Acaulospora-Gigaspora-Glomus* or association of mycorrhiza with NPK fertilizers offered the similar number of pods per plant (8 to 10). Treatment with low doses of NPK (100 Kg NPK ha⁻¹) combined with *Acaulospora-Gigaspora-Glomus* inoculum yielded well (15%) compared to the control and reduces the quantity of chemical fertilizers to be applied. NPK alone or inoculation with *Acaulospora-Gigaspora-Glomus* AMF inoculum improved bean yield by 12%.

Keywords Arbuscular mycorrhizae · NPK fertilizers · Common bean · Acidic soil

✉ Bibich Kirika Ansey
anseykir@gmail.com

¹ Unité de Recherche en Systèmes de Production Végétale, Department of Crops Sciences, Faculty of Agronomy, University of Lubumbashi, PO Box 1825, Lubumbashi, Congo

² Key Laboratory of Crop Germplasm Resource, Department of Agronomy, College of Agriculture and Biotechnology, Zijingang Campus, Zhejiang University, Yuhangtang Road, 310058 Hangzhou, China

³ Unité de Recherche en défense et protection des végétaux, Department of Crops Sciences, Faculty of Agronomy, University of Lubumbashi, PO Box 1825, Lubumbashi, Congo

⁴ Unité de Recherche en Ecologie, Restauration Ecologique et Paysage, Department of Natural Resources Management, Faculty of Agronomy, University of Lubumbashi, PO Box 1825, Lubumbashi, Congo

⁵ Faculty of Faculty of Bioscience Engineering, Department Environment, Campus Schoonmeersen, Ghent University, Valentin Vaerwyckweg 1, 9000 Gent, Belgium

⁶ Faculty of Faculty of Bioscience Engineering, Department Plants and Crops, Campus Schoonmeersen, Ghent University, Valentin Vaerwyckweg 1, 9000 Gent, Belgium

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most cultivated food legumes in several African countries compared to other species of the same genus. Its cultivation covers more than 3 million hectares annually (Yayis et al. 2011). In developing countries such as the Democratic Republic of Congo (DRC), Phaseolus bean is an important source of protein, starch, dietary amino acids and minerals, especially iron and potassium, playing an important role in human daily diet (Meseret and Amin 2014; Sabry et al. 2017).

In the Katanga region, common bean is one of the commonly grown food crops while its production in the entire region does not exceed 10% of the demand (Kanyenga et al. 2012). Thus, imports are needed to cover the gap. Moreover, in this part of the country, the production is not constant as it varies according to the cropping system (Tshibingu et al. 2017). In Haut-Katanga, common bean yields range from 800 to 1500 kg ha⁻¹ for large-scale producers who use improved varieties and chemical fertilizers. However, for small-scale producers, yields range from 200 to 400 kg ha⁻¹ because fields are not generally fertilized and the soil pH ranges from 4 to 5 (Kanyenga et al. 2012; Mufind et al. 2014; Tshibingu et al. 2017). That low common bean yields also would be due to several factors such as deficiency of nitrogen and potassium, high purchase cost of chemical fertilizers, scarcity of organic fertilizers, as well as diseases and insect pests (Mergeai 2010; Meseret and Amin 2014; Tshibangu et al. 2020).

It would be interesting to improve the fertility of these acidic, phosphorus-deficient soils and low in organic matter soils and upgrade their biological activity to increase the yield of common beans in the Katanga region (Tshibangu et al. 2020).

Nitrogen acquisition in bean is achieved through its symbiotic association with nitrogen-fixing *Rhizobium* species (Razakatiana et al. 2020; Pastor-Bueis et al. 2021). If AMF are presents, phosphorus is provided in part (Fortin et al. 2008; Cardoso et al. 2017; Razakatiana et al. 2020). In poor or degraded soils, mycorrhizal symbioses, which are the most ubiquitous, promote nutrient uptake, especially phosphorus, and improve seed yield in common beans (Baslam et al. 2011; Tshibangu et al. 2020). They also provide plants with robustness and resistance to bacterial and fungal pathogens (Harrier and Watson 2004; Baslam et al. 2011; Shukla et al. 2013). The aim of this study was to contribute to increased common bean production in acidic soils (pH 4.2 to 5.5) prevailing in the Upper Katanga region. Mycorrhizal inoculation (AMF) was combined with NPK10-20-10 fertilizer to improve fertilize use efficiency and to assure an acceptable yield level.

Materials and Methods

AMF Inoculum Production

The soils were collected from wild floristic herbaceous communities particularly dominated by: *Thitonia diversifolia* and *Loudetia simplex* (community 1 which had 45 AMF spores 100 g⁻¹ soil), *Panicum maximum*, *Hyperhenia rufa* and *H. diplandra* (community 2: 15 AMF spores 100 g⁻¹ soil); *Imperata cylindrica* and *Cynodon dactylon* (community 3: 22 AMF spores 100 g⁻¹ soil); *Nephrolepis undulata*, *Monosymbium ceresiiforme* and *Bidens oligoflora* (community 4: 33 AMF spores 100 g⁻¹ soil). AMF morphotypes were isolated from these soils, quantified, identified and cultured under *Plantago lanceolata* which is a trap plant in order to increase the number of spores. In general, without considering diversity, after 3 months, the mean density of AMF spores increased from 30 per 100 g of soil to 155 spores per 100 g of soil after *Plantago lanceolata* trap culture. The production of these inocula was done in 30 cm diameter pots containing 15 kg of soil, under a shaded net house installed at the Faculty of Agronomic Sciences of the University of Lubumbashi. AMF inocula were produced during rainy season (November 2018 to January 2019) under the following atmospheric conditions: 18 to 24 °C, a duration of insolation of 12 h with an atmospheric humidity ranging from 50 to 70%. AMF identification was done using the Redecker et al. (2013) key. Based on the above-mentioned soil origins, four types of AMF inocula were obtained and characterized as follows:

- Inoculum from wild floristic herbaceous community 1: community of AMF dominated by *Acaulospora* (*Acaulospora* sp, *A. scrobiculata*, *A. colossica*, *A. delicata*, *Glomus* sp and *Ambispora* sp) with a mean density of 155 spores per 100 g of soil;
- Inoculum wild floristic herbaceous community 2: containing a mean density of 75 spore per 100 g soil⁻¹ of *Gigaspora* (*Gigaspora rosea*, *Gigaspora. albida*, *Gigaspora* sp, *Glomus constrictum*, *Acaulospora scrobiculata*);
- Inoculum wild floristic herbaceous community 3: dominated by *Glomus* (*Glomus* sp, *Glomus constrictum*, *Glomus pubescens*, *Funnelformis* sp, *Acaulospora scrobiculata*, *Gigaspora rosea*) with a density of 90 spore 100 g soil⁻¹;
- Inoculum wild floristic herbaceous community 4: characterized by a strong presence of *Acaulospora-Gigaspora-Glomus* for a density of 120 spore 100 g soil⁻¹.

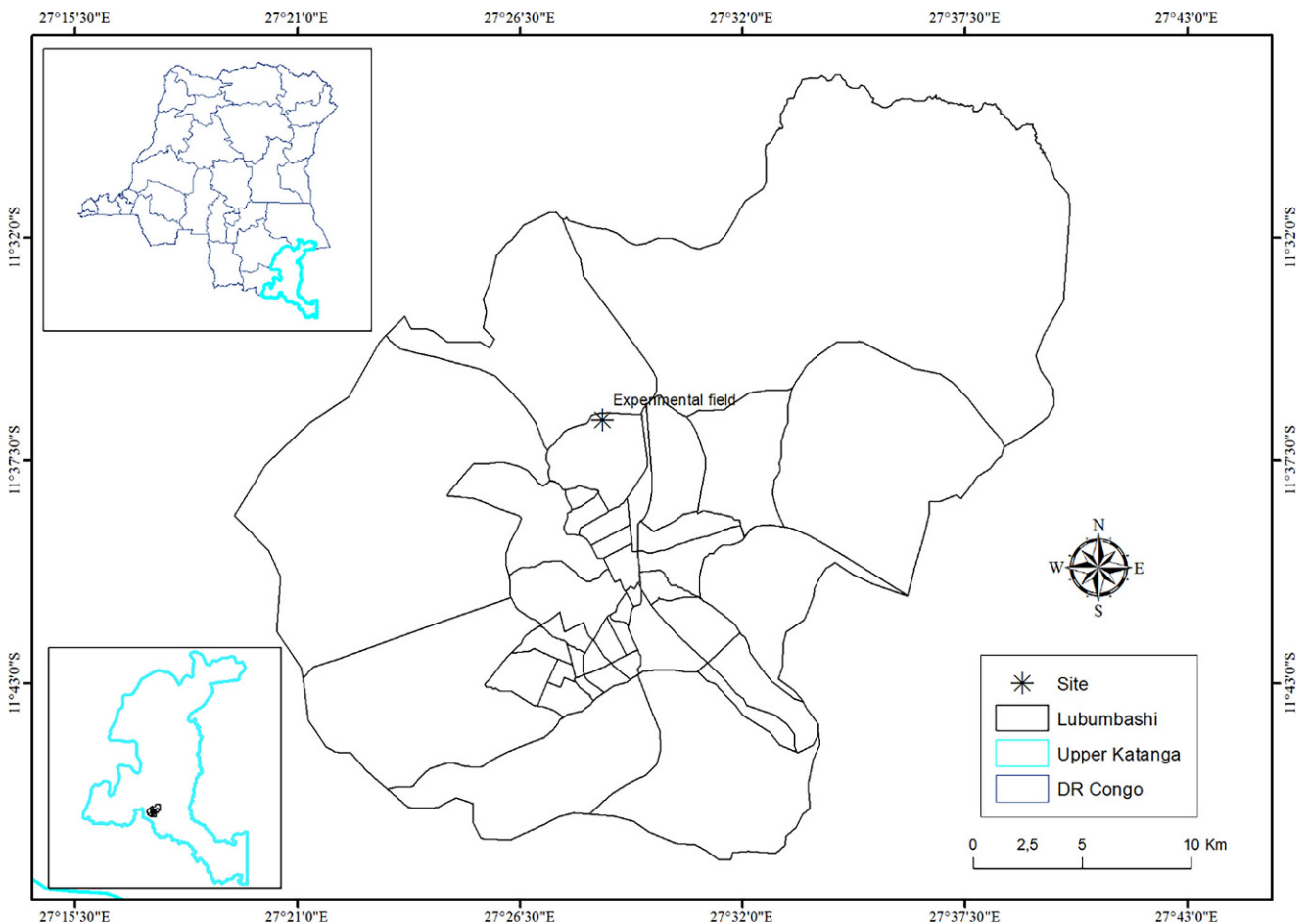


Fig. 1 Study area map

Chemical Fertilizers

The NPK10-20-10 compound fertilizer was applied at a dose of 200 kg ha⁻¹ or 100 kg ha⁻¹ and compared to an unfertilized control; 200 kg/ha refers to the maximum amount of NPK applied to the common bean crop in the Lubumbashi region (Tshibingu et al. 2017). The high level of NPK10-20-10 was chosen regarding what is generally used by great farmers to tackle soil deficiency and, the unfertilized plot was used as a control in accordance with the fact that many smallholder farmers in DRC still believe that leguminous crops doesn't need any fertilization (Mushagalusa et al. 2015).

Bean Variety Grown

The biofortified dwarf variety RWR 2154 of common bean was used for its appreciated characteristics. This variety gives brown seeds with white streaks, is rich in iron and zinc and was supplied by the Harvest plus Project. The number of pods varies between 5 and 11 and maturation occurs 80 to 90 days after sowing.

Experimental Site

Experiment was conducted at the experimental field of Faculty of Agronomic Sciences of University of Lubumbashi at 1259 m altitude at GPS coordinates $-11^{\circ}36'29.7''$ South and $27^{\circ}28'32.0''$ East (Fig. 1).

The soil in which the experiment was conducted is a plinthosol as classified in WRB-2015 (IUSS Working Group WRB. 2015) characterized by dark reddish brown sandy clay with an acidic pH_{H₂O} (4.28). This experimental soil have a good content of organic matter (5.7%) and iron (4.3%). However, the available nitrogen and phosphorus content is low (0.02% and 4.4 mg kg soil⁻¹ respectively). Experimental soil is also characterized by AMF spores density of 45 spores 100 g soil⁻¹). The detailed physical and chemical properties of the experimental soil are shown in Table 1.

Design of Experiment

The experiment was conducted in a 3×5 factorial design with 4 replications. Chemical fertilizer NPK was the main

Table 1 Chemical properties of experimental soil

Location	Soil color	pH _{water}	Organic carbon (%)	Total nitrogen (%)	Total phosphorus (mg kg ⁻¹)	Available phosphorus (mg kg ⁻¹)	Available potassium cmol (+) kg soil ⁻¹	Total iron (%)	ECC cmol (+) kg ⁻¹ soil
S 11°36'29.7"	Dark reddish brown (5YR 3/2)	4.28	3.35	0.02	66.5	4.45	0.54	4.32	6.23
E 27°28'32.0"									

factor and was applied at 3 levels: 100 kg ha⁻¹, 200 kg ha⁻¹ and an unfertilized control. This was combined with AMF inoculums (secondary factor) containing predominantly *Acaulospora* (inoculum 1); *Gigaspora*-dominated inoculum (inoculum 2); *Glomus*-dominated inoculum (inoculum 3) and *Acaulospora-Gigaspora-Glomus*-dominated inoculum (inoculum 4) as well as uninoculated plots; leading to a total of 60 plots. That constitute 15 treatments presented as follows:

- T0: control;
- T1: (*Acaulospora*)
- T2: (*Gigaspora*)
- T3: (*Glomus*)
- T4: (*Acaulospora-Gigaspora-Glomus*)
- T5: 100 kg NPK ha⁻¹
- T6: (*Acaulospora*) + 100 kg NPK ha⁻¹
- T7: (*Gigaspora*) + 100 kg NPK ha⁻¹
- T8: (*Glomus*) + 100 kg NPK ha⁻¹
- T9: (*Acaulospora-Gigaspora-Glomus*) + 100 kg NPK ha⁻¹
- T10: 200 kg NPK ha⁻¹
- T11: (*Acaulospora*) + 200 kg NPK ha⁻¹
- T12: (*Gigaspora*) + 200 kg NPK ha⁻¹
- T13: (*Glomus*) + 200 kg NPK ha⁻¹
- T14: (*Acaulospora-Gigaspora-Glomus*) + 200 kg NPK ha⁻¹

The plots had a surface of 1 m². Sowing were at a distance of 40 × 20 cm, i.e. 18 plants per m². The experiment was conducted between February to May 2019.

Data Collection

Chlorophyll content in leaves was quantified using a KONICA MINOLTA chlorophyll meter on the third photosynthetically active basal leaf. The total height of the plants as well as the number of leaves per plant were evaluated at 60 days after sowing. Spore density of AMF was assessed at 60 days by wet sieving a soil sample on a 45 μm mesh over a 1 mm mesh, followed by centrifugation and addition of sucrose solution (Walker et al. 2006).

The frequency of root colonization by AMF was determined according to the method of Phillips and Hayman (1970). The procedure consists of clearing roots with 10% KOH heated in a water bath for 30 min and stained with

methylene blue. AMF structures showing colonization are: hyphae, vesicles and arbuscules. Frequency of colonization was expressed as the number of roots bearing one of these structures expressed as % of the total number of roots observed.

Incidence of root rot caused by the common bean fly *Ophiomyia phaseoli* was assessed by counting the number of attacked plants out of the total number of plants in the plot multiplied by 100. The number of pods per plant, seed yield and phosphorus content in seeds were also assessed by colorimetric of ash extraction following the Van Ranst et al. (1999) method.

Data Analysis

The analysis of variance (ANOVA) was applied in the factorial design to compare means between chemical fertilization levels, AMF inoculation and interactions. Data with a non-normal distribution were log-transformed prior to this analysis, following the Kolmogorov method. Separation of means was done by the Tukey HSD test at the $P < 0.05$ significance level. Minitab 16 software was used for statistical analysis.

Results

Growth Parameters and Incidence of Bean Fly

Plant height, number of leaves per plant, leaves chlorophyll content and incidence of bean fly were recorded at 60 days after sowing (Table 2). Plant height was significantly influenced by NPK fertilizer, the highest average (34 ± 3.6 cm) was obtained on plots received a fertilization with 200 kg NPK ha⁻¹ while the control treatment showed a low mean plant height (21 ± 21 cm). The treatment with 100 kg NPK ha⁻¹ with a mean plant height of 27 ± 2.7 cm scored intermediately.

The inoculum had no significant effect on plant height at this development stage. It appears from the interaction that the highest height is obtained with 200 kg NPK ha⁻¹ alone (40 ± 1 cm) or with the fertilization of 200 kg combined with *Acaulospora-Gigaspora-Glomus* AMF (35 ± 2 cm). Moreover, the lowest height was obtained on the control (17 ± 1 cm), others treatments gave intermediate height

Table 2 Plant height, number of leaves, leaves chlorophyll content and attack incidence of common bean at 60 days after seedling

Treatments	Plant height (cm)	Number of leaves	Leaves chlorophyll content	Attack incidence (%)
Chemical Fertilizer (NPK) effect				
Without chemical fertilizer	21.30±21.30 c	6±0.95 c	37.43±1.32 a	26.54±7.29 a
100kg NPK ha ⁻¹	27.05±2.74 b	7±1.12 b	37.60±1.75 a	23.39±7.28 b
200kg NPK ha ⁻¹	34.35±3.64 a	9±0.99 a	38.00±2.85 a	21.88±7.83 c
AMF effect				
Without AMF	27.83±9.74 A	8±1.85 A	37.30±2.57 A	29.70±8.40 A
<i>Acaulospora</i> inoculum	27.08±6.77 A	7±1.56 A	37.62±1.62 A	24.66±8.04 AB
<i>Gigaspora</i> inoculum	27.58±4.01 A	8±0.94 A	37.75±1.21 A	23.46±6.59 AB
<i>Glomus</i> inoculum	26.08±5.21 A	7±1.13 A	37.65±1.88 A	24.16±6.17 AB
<i>Acaulospora-Gigaspora-Glomus</i> inoculum	29.25±4.47 A	8±1.20 A	38.06±2.84 A	17.70±3.72 B
Interaction fertilizer × AMF				
Control	17.75±1.70 h	5±0.64 d	36.91±0.94 a	32.63±5.09 a
<i>Acaulospora</i> inoculum	20.25±3.30 gh	7±0.90 cd	37.91±0.24 a	20.82±3.73 ab
<i>Gigaspora</i> inoculum	23.50±2.38 fgh	7±0.34 cd	37.16±0.70 a	22.44±11.40 ab
<i>Glomus</i> inoculum	20.75±1.70 gh	7±1.07 abcd	37.30±1.26 a	20.97±4.18 ab
<i>Acaulospora-Gigaspora-Glomus</i> inoculum	24.25±2.87 fg	8±0.57 abcd	37.89±2.65 a	20.10±1.35 ab
100kg NPK ha ⁻¹	25.75±1.70 efg	8±0.66 abcd	36.79±0.95 a	30.10±4.17 ab
100kg NPK ha ⁻¹ + <i>Acaulospora</i> inoculum	26±1.82 defg	6±1.56 d	37.37±2.93 a	29.08±10.43 ab
100kg NPK ha ⁻¹ + <i>Gigaspora</i> inoculum	27.5±2.51 cdef	7±0.44 abcd	37.92±1.06 a	26.17±1.79 ab
100kg NPK ha ⁻¹ + <i>Glomus</i> inoculum	25.75±3.20 efg	7±1.37 bcd	37.94±2.49 a	29.82±7.05 ab
100kg NPK ha ⁻¹ + <i>Acaulospora-Gigaspora-Glomus</i> inoculum	30.2±2.21 bcde	7±0.77 cd	37.98±1.09 a	17.55±3.16 ab
200kg NPK ha ⁻¹	40.00±1.82 a	10±0.48 a	38.20±4.57 a	26.38±13.75 ab
200kg NPK ha ⁻¹ + <i>Acaulospora</i> inoculum	33.25±2.06 bc	8±0.99 abc	37.60±0.89 a	24.07±8.24 ab
200kg NPK ha ⁻¹ + <i>Gigaspora</i> inoculum	31.75±1.25 bcd	8±0.80 abc	38.16±1.74 a	21.78±3.30 ab
200kg NPK ha ⁻¹ + <i>Glomus</i> inoculum	31.75±2.36 bcd	8±1.09 abcd	37.71±2.20 a	21.70±2.83 ab
200kg NPK ha ⁻¹ + <i>Acaulospora-Gigaspora-Glomus</i> inoculum	35.00±2.58 ab	9±0.56 ab	38.33±4.61 a	15.46±4.95 b

Means that share no letters in each column within a group are significantly different after Tukey HSD test ($P < 0.05$)

means. Number of leaves were also significantly influenced by NPK fertilizer, which 9±0.99 leaves after the application of 200kg NPK ha⁻¹ while the control had 6±0.9 leaves per plant. The best treatment was obtained with 200kg NPK ha⁻¹ alone and with 200kg NPK ha⁻¹ in combination with *Acaulospora-Gigaspora-Glomus* AMF inoculum (9±0.5 to 10±0.4 leaves per plant) compared to uninoculated control, which a 5±0.6 leaves. Leaves chlorophyll was not influenced by fertilization, AMF inoculum and for their interactions. The incidence of bean fly attack showed that the 200kg NPK ha⁻¹ treatment were less attacked (22±7.8%) than the control plots which were characterized by a high incidence (27±7.29%). AMF had significant effects on the incidence rate, which ranged from 18±3.7 to 25±8.04% in contrast to the uninoculated treatment which had a high incidence of 30±8.4%. The interaction between NPK compound fertilizer with AMF inoculum showed a much better bean fly attack on the untreated plots (33±5.09%) vs 15±4.9% observed on the

200kg NPK ha⁻¹ treatment associated with *Acaulospora-Gigaspora-Glomus* AMF.

Spore Density and Root Colonization Frequency by AMF

The NPK compound fertilizer had strong influence on both spore density and root colonization frequency (Table 3 below). Higher level of chemical fertilizer (200kg ha⁻¹), decreased the spore density and root colonization frequency with a mean of 34 spores 100g soil⁻¹ and 44% respectively, as compared to the control, which showed 83 spores 100g soil⁻¹ with a root colonization frequency of 72%.

AMF adding significantly increased spore density (60±25 to 63±29 100g soil⁻¹) and root colonization frequency (58±16 to 60±18%) in contrast to the control which had a mean of 37±15 spores 100g soil⁻¹ and 40±13% of root colonization frequency. Interaction between AMF and NPK showed a high spore density on the inoculated treatments (86±20 to 98±12 spores 100g soil⁻¹)

Table 3 AMF spore density and root colonization frequency of common bean at 60 days after seedling

Treatments	AMF spore density 100 g soil ⁻¹	Root colonization frequency (%)
Chemical Fertilizer (NPK) effect		
Without chemical fertilizer	83 ± 23 a	72 ± 10 a
100 kg NPK ha ⁻¹	52 ± 15 b	52 ± 9 b
200 kg NPK ha ⁻¹	34 ± 9 c	40 ± 8 c
AMF effect		
Without AMF	37 ± 15 B	40 ± 13 B
<i>Acaulospora</i> inoculum	60 ± 25 A	60 ± 18 A
<i>Gigaspora</i> inoculum	61 ± 24 A	59 ± 14 A
<i>Glomus</i> inoculum	63 ± 29 A	58 ± 16 A
<i>Acaulospora-Gigaspora-Glomus</i> inoculum	62 ± 29 A	59 ± 14 A
Interaction fertilizer × AMF		
Control	51 ± 12 bcd	60 ± 8 cd
<i>Acaulospora</i> inoculum	86 ± 24 a	80 ± 8 a
<i>Gigaspora</i> inoculum	86 ± 20 a	69 ± 6 bc
<i>Glomus</i> inoculum	98 ± 12 a	75 ± 4 ab
<i>Acaulospora-Gigaspora-Glomus</i> inoculum	94 ± 18 a	74 ± 9 ab
100 kg NPK ha ⁻¹	36 ± 13 cde	40 ± 4 gh
100 kg NPK ha ⁻¹ + <i>Acaulospora</i> inoculum	59 ± 9 b	56 ± 8 d
100 kg NPK ha ⁻¹ + <i>Gigaspora</i> inoculum	61 ± 6 b	55 ± 7 de
100 kg NPK ha ⁻¹ + <i>Glomus</i> inoculum	55 ± 8 bc	53 ± 10 def
100 kg NPK ha ⁻¹ + <i>Acaulospora-Gigaspora-Glomus</i> inoculum	52 ± 22 bcd	59 ± 6 cd
200 kg NPK ha ⁻¹	23 ± 4 e	33 ± 6 h
200 kg NPK ha ⁻¹ + <i>Acaulospora</i> inoculum	36 ± 4 de	43 ± 10 fgh
200 kg NPK ha ⁻¹ + <i>Gigaspora</i> inoculum	37 ± 5 cde	39 ± 3 gh
200 kg NPK ha ⁻¹ + <i>Glomus</i> inoculum	36 ± 7 cde	41 ± 3 gh
200 kg NPK ha ⁻¹ + <i>Acaulospora-Gigaspora-Glomus</i> inoculum	40 ± 11 cde	46 ± 9 efg

Means that share no letters in each column within a group are significantly different after Tukey HSD test ($P < 0.05$)

while the 200 NPK ha⁻¹ treatment gave only 23 ± 4 spores 100 g soil⁻¹. The 200 kg NPK ha⁻¹ treatment significantly reduced the root colonization frequency by AMF (33 ± 6%) compared to the *Acaulospora*, *Glomus* or *Acaulospora-Gigaspora-Glomus* inocula without inoculation, without NPK, which offered 74 ± 9 to 80 ± 8%. The others treatments showed intermediate values.

Yield Parameters

Table 4 shows that the NPK compound fertilizer had significant effects on the number of pods per plant, yield, and grains phosphorus content. The number of pods varied between 7 ± 1 and 8 ± 1 on 100 kg NPK or 200 kg ha⁻¹ than untreated plots (5 ± 1.2 pods per plant). AMF inoculum (*Acaulospora-Gigaspora-Glomus*) also had significant effects on the pod number with (9 ± 1) per plant compared to others treatments which had 6 ± 1 to 7 ± 1 pods per plant. Treatments with 200 kg NPK ha⁻¹ alone or combined to *Acaulospora-Gigaspora-Glomus* inoculum gave the same number of pods per plant (8 to 10) as treatments with 100 kg NPK ha⁻¹ combined with *Acaulospora-Gigaspora-Glomus* or *Acaulospora-Gigaspora-Glomus* inoculum alone. However, the lowest number of pods per plant was obtained on the control (5 pods) while the other treatments gave an intermediate number of pods per plant (6 to 7 pods).

The highest yield (469.13 ± 70.65 kg ha⁻¹) was obtained by the 200 kg NPK ha⁻¹ treatment while the untreated plots recorded the lowest yield (365.70 ± 46.41 kg ha⁻¹). The treatment 100 kg ha⁻¹ gave an intermediate yield (425.56 ± 38.37 kg ha⁻¹). AMF significantly influenced yield with *Acaulospora-Gigaspora-Glomus* offering 504.01 ± 54.58 kg ha⁻¹ against the control which gave 386.93 ± 28.12 kg ha⁻¹.

Interactions shows that the best yield was obtained by using 200 kg NPK ha⁻¹ alone or 200 kg NPK ha⁻¹ combined with *Acaulospora-Gigaspora-Glomus* AMF with means varying respectively from 524.48 ± 22.67 kg ha⁻¹ to 569.43 ± 28.63 ha⁻¹ against 348.83 ± 21.99 ha⁻¹ obtained on the control plots. The high grains phosphorus content was offered by 100 kg or 200 kg NPK ha⁻¹ treatment (0.35 ± 0.29 to 0.36 ± 0.04%). AMF inoculum also had significant effects on the phosphorus grain content which was high on plots treated with *Acaulospora-Gigaspora-Glomus* (0.37 ± 0.02) compared to the uninoculated plots which contained less Phosphorus (0.32 ± 0.04%); others treatments showed similar phosphorus grains contents. Interaction showed that the highest phosphorus grains content was obtained on *Acaulospora-Gigaspora-Glomus* treatment (0.40 ± 0.4%); this was significantly higher than the control (0.28 ± 0.2%).

Discussion

Growth Parameters and Incidence of the Fly

The plant height, the number of leaves per plant and incidence of the fly were significantly influenced by NPK fertilizer more than by AMF inocula. The direct effect of NPK fertilizer was stronger than this of AMF inocula; as expressed by higher plant height and number of leaves.

Table 4 Number of pods per plant, yield and grains phosphorus content

Treatments	Pods per plant	Yield (kg per ha)	Grain phosphorus content
Chemical Fertilizer (NPK) effect			
Without chemical fertilizer	5 ± 1.29 b	365.70 ± 46.41 c	0.30 ± 0.03 b
100 kg NPK ha ⁻¹	7 ± 1 ab	425.56 ± 38.37 b	0.35 ± 0.29 a
200 kg NPK ha ⁻¹	8 ± 1 a	469.13 ± 70.65 a	0.36 ± 0.04 a
AMF effect			
Without AMF	6 ± 2 B	386.93 ± 28.12 C	0.32 ± 0.04 B
<i>Acaulospora</i> inoculum	6 ± 1 B	403.21 ± 25.05 B	0.33 ± 0.04 AB
<i>Gigaspora</i> inoculum	7 ± 1 B	419.23 ± 15.65 B	0.33 ± 0.04 AB
<i>Glomus</i> inoculum	6 ± 1 B	404.27 ± 78.96 B	0.33 ± 0.04 AB
<i>Acaulospora-Gigaspora-Glomus</i> inoculum	9 ± 1 A	504.015 ± 54.58 A	0.37 ± 0.02 A
Interaction fertilizer × AMF			
Control	5 ± 1 g	348.83 ± 21.99 g	0.28 ± 0.28 e
<i>Acaulospora</i> inoculum	6 ± 1 defg	382.42 ± 25.63 efg	0.38 ± 0.38 c
<i>Gigaspora</i> inoculum	7 ± 1 cdef	408.62 ± 15.77 defg	0.39 ± 0.39 b
<i>Glomus</i> inoculum	5 ± 1 fg	369.44 ± 19.72 fg	0.39 ± 0.39 b
<i>Acaulospora-Gigaspora-Glomus</i> inoculum	8 ± 1 abcd	469.20 ± 18.85 bcd	0.40 ± 0.40 a
100 kg NPK ha ⁻¹	6 ± 1 cdefg	438.50 ± 36.01 cde	0.33 ± 0.33 h
100 kg NPK ha ⁻¹ + <i>Acaulospora</i> inoculum	6 ± 1 defg	410.90 ± 18.30 def	0.27 ± 0.27 i
100 kg NPK ha ⁻¹ + <i>Gigaspora</i> inoculum	7 ± 0 cdef	418.50 ± 9.87 cdef	0.29 ± 0.29 g
100 kg NPK ha ⁻¹ + <i>Glomus</i> inoculum	6 ± 1 efg	386.50 ± 27.48 efg	0.28 ± 0.28 h
100 kg NPK ha ⁻¹ + <i>Acaulospora-Gigaspora-Glomus</i> inoculum	9 ± 0 ab	473.40 ± 34.32 bc	0.35 ± 0.35 d
200 kg NPK ha ⁻¹	8 ± 1 abc	524.48 ± 22.67 ab	0.39 ± 0.39 b
200 kg NPK ha ⁻¹ + <i>Acaulospora</i> inoculum	7 ± 1 cdf	416.31 ± 20.65 cdef	0.33 ± 0.33 e
200 kg NPK ha ⁻¹ + <i>Gigaspora</i> inoculum	7 ± 1 cdef	430.57 ± 15.17 cde	0.33 ± 0.33 e
200 kg NPK ha ⁻¹ + <i>Glomus</i> inoculum	7 ± 1 bcde	404.86 ± 30.31 efg	0.32 ± 0.32 f
200 kg NPK ha ⁻¹ + <i>Acaulospora-Gigaspora-Glomus</i> inoculum	10 ± 1 a	569.43 ± 28.63 a	0.38 ± 0.38 c

Means that share no letters in each column within a group are significantly different after Tukey HSD test ($P < 0.05$)

Previous studies have also demonstrated that plants might directly absorb enough nutrients (phosphorus and nitrogen) without help from AMF symbiosis following chemical fertilizer, and then, plants decrease their dependence on belowground AMF symbiosis (Kamlesh and Smritikana 2018; Trejo et al. 2021).

AMF had no significant effect on plant height, number of leaves per plant, and leaves chlorophyll content. This could be attributed to rhizobia symbiosis on roots that provides nitrogen to the plant that would promote height, number of leaves and leaves chlorophyll content (Kinany et al. 2019; Wawan et al. 2020; Jekabsone et al. 2022).

The treatment of 200 kg NPK ha⁻¹ alone or combined to *Acaulospora-Gigaspora-Glomus* inoculum offered robust plants and less incidence of fly (15%) as compared to uninoculated treatment whose highly impacted by fly 32.6%. These results shows that AMF inoculum enhances plant tolerance against the bean fly pest; Harrier and Watson (2004); Douds et al. (2005); Abdel-fattah et al. (2010); Smith et al. (2011); Shukla et al. (2013) and Thanni et al. (2022) showed better nutrients uptake of NPK fertilized

and AMF inoculated plots thereby increase the plant defense against pests than uninoculated plants, which showed a high impact of pests.

AMF Spore Density and Root Colonization Frequency

NPK fertilizer significantly reduced the spore density (34 ± 9 against 83 ± 23 spores 100 g soil⁻¹) and the root colonization frequency by AMF (40 ± 8 against $72 \pm 10\%$); however, these parameters increased with AMF inoculum. NPK fertilizer contains available nitrogen, phosphorus and potassium which can be directly assimilated by plants, which reduce AMF symbiosis and its infective propagules. This was also reported by Tshibangu et al. (2020) who found that chemically fertilized common bean were less colonized than unfertilized plants.

Shukla et al. (2013); Abdel-fattah et al. (2016); Mukhongo et al. (2017) showed that although AMFs are ubiquitous in nature, it is often important to bring the exogenous strains as inoculum to the growth environment of crops to increase number of spores as well as root colonization frequency

which would justify the fact that the uninoculated plants showed low spore density and root colonization frequency (37 ± 15 spores 100 g soil^{-1} and $40 \pm 13\%$ respectively) compared to those inoculated with AMF.

Yield Parameters and Grains Phosphorus Content

The number of pods per plant was high (8 to 10 pods) for $200 \text{ kg NPK ha}^{-1}$ treatments than those inoculated with *Acaulospora-Gigaspora-Glomus* without NPK fertilizer as well as the combination *Acaulospora-Gigaspora-Glomus* with 100 or $200 \text{ kg NPK ha}^{-1}$, contrary to the control which had 5 pods per plant. Other treatments gave an intermediate number of pods. This equality of pod numbers, especially when comparing treatments inoculated with *Acaulospora-Gigaspora-Glomus* AMF strains treatments with 100 kg or $200 \text{ kg NPK ha}^{-1}$, would be explained by good spore density and high root colonization frequency resulting in an increase of exploitable rhizospheric zone for nutrient uptake (Smith and read 2008; Li et al. 2009; Smith et al. 2011; Giovannini et al. 2020). Good mycorrhizal activity makes phosphorus available for plants, which is a major element involved in several metabolic processes in both nucleic acid formation and fruiting (Li et al. 2009; Sabry et al. 2017; Tshibangu et al. 2021).

Inoculation also resulted in a 12% increase in yield compared to the control, confirming here that AMF have demonstrated their role as biofertilizers (Mukhongo et al. 2017; Begum et al. 2019); these results are confirmed by Cozzolino et al. (2013) and Halim et al. (2015) who demonstrate that inoculation with AMF leads to a better biological soil fertility and increases crop production by about 10%. The highest yield obtained during this experiment being 569 kg ha^{-1} , still represents a low average compared to that obtained by large-scale producers (800 kg ha^{-1}). This could be explained by the low number of seeds per seed hole (one seed per seed hole) reducing density of plants per m^2 , the low rainfall (121.4 mm during experiment period), compared to the requirements of the crop which varies between 300 to 500 mm . On the other hand, acidity of experimental soil pH H_2O (4.2), will have also limits the yield level since the optimal pH goes from 6.0 to 6.8 (Alemu 2017). The presence of bean fly although less severe on the best treatment (15% of attack) would also contributed to this low yield. The treatment of 100 Kg of NPK ha^{-1} combined with *Acaulospora-Gigaspora-Glomus* inoculum recorded a yield level of $473.40 \pm 34.32 \text{ kg ha}^{-1}$ and even stimulated the AMF symbiosis. The installation of AMF symbiosis is followed by an increase in mycelium biomass on and in the roots; this allows the plant to make highly nutrients uptake and result in a yield equal to that obtained on treatments with the recommended doses of $200 \text{ NPK kg/ha}^{-1}$ fertilizers; thus, AMF inoculation allows to decrease the quantity of NPK

fertilizers; these results corroborate with those of Youssef et al. (2017) and Tshibangu et al. (2020) who reach same conclusions.

AMF inoculation resulted in higher phosphorus grains content in contrast to uninoculated treatments. AMF offer several benefits to 80% of species, including beans, particularly improvement of phosphorus uptake (Baslam et al. 2011; Hashem 2015; Tshibangu et al. 2020, 2021; Razakaitiana et al. 2020; Trejo et al. 2021).

Conclusion

The aim of this work was to contribute to the increase of common bean production in the dominant acidic soils in the Lubumbashi region with mean value of pH 4.2. Application of $200 \text{ Kg NPK ha}^{-1}$ compound fertilizer reduces AMF spore density and colonization frequencies on roots, plant height and leaf number varied more with chemical fertilization than AMF. The inoculation of common bean with AMF also entrances it tolerance against the bean fly; $200 \text{ kg NPK ha}^{-1}$ in the bean crop or $100 \text{ kg NPK ha}^{-1}$ combined with *Acaulospora-Gigaspora-Glomus* inoculum gives similar yields, demonstrating here the implication of AMF symbiosis as a biofertilizer making chemical fertilizers efficient.

Acknowledgements We thank the VLIRUOS Project for financing this research, both for the implementation of the experiment and for the laboratory analyses.

Conflict of interest Bibich Kirika Ansey, Audry Tshibangu Kazadi, Jonas Lwalaba wa Lwalaba, Mick Assani Bin Lukangila, Mylor Ngoy Shutcha, Geert Baert, Geert Haesaert and Robert-Prince Mukobo Mundende declare that they have no competing interests.

References

- Abdel-Fattah GM, El-Haddad SA, Hafez EE, Rashad YM (2010) Induction of defense responses in common bean plants by arbuscular mycorrhizal fungi. *Microbiol Res* 166(4):268–281
- Abdel-Fattah GM, Hassan Rabie G, Shaaban Lamis D, Metwally Rabab A (2016) The impact of the arbuscular mycorrhizal fungi on growth and physiological parameters of cowpea plants grown under salt stress conditions. *Int J Appl Sci Biotechnol* 4(3):372–379
- Alemu H (2017) Breeding Common Bean (*Phaseolus Vulgaris* L.) Genotypes for acidic soil tolerance. *Int. J. Adv. Res. Publ.* 1(3):39–46
- Baslam M, Pascuaal I, Sanchez-Diaz M, Erro J, Garcia JM, Goicoechea N (2011) Improvement of nutritional quality of greenhouse-grown lettuce by arbuscular mycorrhizal fungi is conditioned by the source of phosphorus nutrition. *J Agric Food Chem* 59:11129–11140
- Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang L (2019) Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Front Plant Sci* 10:1068

- Cardoso EJBN, Nogueira MA, Zangoro W (2017) Importance of mycorrhizae in tropical soils. In: Diversity and benefits of microorganisms from the tropics, vol 11, pp 245–267
- Cozzolino V, Di Meo V, Piccolo A (2013) Effect of arbuscular mycorrhizal fungi applications on maize production and phosphorus availability. *J Geochem Explor* 129:40–44
- Douds DD, Nagahashi G Jr, Pfeffer PE, Kayser WM, Reider C (2005) On-farm production and utilisation of arbuscular mycorrhizal fungus inoculum. *Can J Plant Sci* 85:15–21
- Fortin JA, Plenchette C, Piche Y (2008) Les mycorrhizes : la nouvelle révolution verte. Editions multimondes, Multi Mondes
- Giovannini L, Palla M, Agnolucci M, Avio L, Sbrana C, Turrini A, Ciovannetti M (2020) Arbuscular mycorrhizal fungi and associated microbiota as plant biostimulants: research strategies for the selection of the best performing inocula. *Agronomy* 10(1):106
- Halim, Arma MJ, Rembon FS, Reman (2015) Impact of mycorrhiza fungi from grassland rhizosphere and liquid organic fertilizer to the growth and yield of sweet corn on ultisols in South Konawe, Indonesia. *Agric For Fish* 4(5):209–215
- Harrier LA, Watson CA (2004) The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. *Pest Manag Sci* 60:149–157
- Hashem MM (2015) Impact of inoculation with arbuscular mycorrhizal, phosphate solubilizing bacteria and soil yeast on growth, yield and phosphorus content on onion plants. *Int J Soil Sci* 10(2):93–99
- IUSS Working Group WRB. (2015) World reference base for soil resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps. World soil resources reports, vol 106. FAO, Rome
- Jekabsons A, Andersone-Ozola U, Karlsons A, Neicenece L, Romanovs M, Ievinsh G (2022) Dependence on nitrogen availability and rhizobial symbiosis of different accessions of trifolium fragiferum, a crop wild relative legume species, as related to physiological traits. *Plants* 11(9):1141
- Kamlesh KY, Smritikana S (2018) Biofertilizers, impact on soil fertility and crop productivity under sustainable agriculture. *Environ Ecol* 37(1):89–93
- Kanyenga LA, Funny BC, Ngoie LM, Mudibu J, Tshilenge LL, Kalonji MA (2012) Yield performance and resistance to angular leaf spot disease in bean (*Phaseolus vulgaris* L.) five agro-ecological zones of Katanga, Democratic republic of Congo. *Int J Res Plant Sci* 2(1):16–22
- Kinany E, Achbani E, Faggroud M, Ouahmane R, El Hilali A, Haggoud A, Bouamri R (2019) Effect of organic fertilizer and commercial arbuscular mycorrhizal fungi on the growth of micropropagated date palm cv. Feggouss. *J Saudi Soc Agric Sci* 18:411–417
- Li H, Shen J, Zhang F, Marschner P, Cawthray G, Rengei Z (2009) Phosphorus uptake and rhizosphere properties of intercropped and monocropped maize, faba, bean, and white lupin in acidic soil. *Biol Fertil Soils* 46:79–91
- Mergeai G (2010) L'agriculture en tant que moteur de la croissance pour lutter contre la pauvreté : potentialités et contraintes de l'agriculture de conservation pour lutter contre la pauvreté rurale en Afrique Sub-saharienne. *Tropicultura* 28(3):129–132
- Meseret T, Amin M (2014) Effect of different phosphorus fertilizer rates on growth, dry matter yield and yield components of common bean (*Phaseolus vulgaris* L.). *World J Agric Res* 2(3):88–92
- Mufind KM, Tshala UJ, Kitabala MA, Kimuni NL (2014) Réponse de huit variétés de haricot commun (*Phaseolus vulgaris* L.) à la fertilisation minérale dans la région de Kolwezi, Lualaba (RD. Congo). *J Appl Biosci* 111:10894–10904
- Mukhongo RW, Tumuhairwe JB, Ebanyat P, AbdelGadir AH, Thuita M, Masso C (2017) Mycorrhizal technology for improving yield production of common bean plants. *Int J Appl Sci Biotechnol* 4(2):191–197
- Mushagalusa BA, Momba NJ, Kasanda MN, Nkulu MFJ (2015) Caractéristiques de l'agriculture familiale dans quelques villages de Kipushi: Enjeux et perspectives pour la sécurité alimentaire. *Int J Innov Appl Stud* 10:1134–1143
- Pastor-Bueis R, Jiménez-Gómez A, Barquero M, Mateos PF, González-Andrés F (2021) Yield response of common bean to co-inoculation with rhizobium and pseudomonas endophytes and microscopic evidence of different colonized spaces inside the nodule. *Eur J Agron* 122:126–187
- Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* 55(1):158–161
- Razakatiana ATE, Trap J, Baohanta RH, Raheirimandimby M, Le Roux C, Duponnois R, Ramanankierana H, Becquer T (2020) Benefits of dual inoculation with arbuscular mycorrhizal fungi and rhizobia on *Phaseolus vulgaris* planted in a low-fertility tropical soil. *Pedobiologia* 83:150685
- Redecker D, Schübler A, Stockinger H, Stürmer SL, Morton JB, Walker C (2013) An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* 23:515–531
- Sabry MY, Gamal SR, Salama AEE (2017) Effect of phosphorus sources and arbuscular mycorrhizal inoculation on growth and productivity of snap bean (*Phaseolus vulgaris* L.). *Gesunde Pflanz* 69:139–148
- Shukla A, Vyas D, Amuradha J (2013) Soil depth: an overriding factor for distribution of arbuscular mycorrhizal fungi. *J Soil Sci Plant Nutr* 13(1):23–33
- Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London
- Smith SE, Jakobsen I, Gronlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots has important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiol* 156(3):1050–1057
- Thanni B, Merckx R, De Bauw P, Boeraeve M, Peeters G, Hauser S, Honnay O (2022) Spatial variability and environmental drivers of cassava-arbuscular mycorrhiza fungi (AMF) associations across Southern Nigeria. *Mycorrhiza* 32:1–3
- Trejo D, Sangabriel-Cone W, Gavito-Pardo ME, Banuelos J (2021) Mycorrhizal inoculation and chemical fertilizer interactions in pineapple under fields conditions. *Agriculture* 11:934
- Tshibangu KA, Ngoy SM, Baert G, Haesaert G, Mukobo MRP (2020) Effect of soil properties on arbuscular mycorrhizae fungi (AMF) activity and assessment of some methods of common bean (*Phaseolus vulgaris* L.) inoculation in Lubumbashi Region (DR Congo). *Sch Bull* 6(8):198–207
- Tshibangu KA, Lwalaba LJ, Kirika AB, Mavungu MJ, Manda KG, Iband KM, Baert G, Haesaert G, Mukobo MRP (2021) Effect of phosphorus and arbuscular mycorrhizal fungi (AMF) inoculation on growth and productivity of maize (*Zea mays* L.) in a tropical ferralsol. *Gesunde Pflanz* 74:159–165
- Tshibingu IM, Assani BLM, Maloba IMM, Lubobo KA (2017) Responses of some genotypes of bio-fortified climbing common bean (*Phaseolus vulgaris* L.) to climatic conditions of three agro-ecological zones, southern DR Congo. *Net J Agric Sci* 5(3):79–84
- Van Ranst E, Verloo M, Demeyer A, Pauwels MJ (1999) Manual of the soil chemistry and fertility laboratory-analytical methods for soils and plants, equipment and management of consumables vol 835
- Walker C, Mize CW, Mc Nabb HS Jr (2006) Populations of endogonaceous fungi at two locations in central Iowa. *Can J Bot* 60:2518–2529

- Wawan S, Bram B, Slamet H, Himawan BA, Kisey Bina H (2020) Effect of arbuscular mycorrhizal fungi and NPK fertilizer on roots growth and nitrate reductase activity of coconut. *J Agron* 19:46–53
- Yayis R, Setegn G, Hatamu Z (2011) Genetic variation for drought resistance in small red seed common bean genotypes. *Afr Crop Sci J* 19(4):303–311
- Youssef SM, Gamal SR, Salama A, Elhady A (2017) Effect of phosphorus sources and arbuscular mycorrhizal inoculation on growth and productivity of snap bean (*Phaseolus vulgaris* L.). *Gesunde Pflanz* 69:139–148

Springer Nature oder sein Lizenzgeber (z.B. eine Gesellschaft oder ein*e andere*r Vertragspartner*in) hält die ausschließlichen Nutzungsrechte an diesem Artikel kraft eines Verlagsvertrags mit dem/den Au-

tor*in(nen) oder anderen Rechteinhaber*in(nen); die Selbstarchivierung der akzeptierten Manuskriptversion dieses Artikels durch Autor*in(nen) unterliegt ausschließlich den Bedingungen dieses Verlagsvertrags und dem geltenden Recht.

Bibich Kirika Ansey PhD student, Faculty of Agronomy, Department of Crops Sciences, Mycorrhiza Laboratory, University of Lubumbashi, Democratic Republic of the Congo; M.Sc. in crop production (2018); agronomist engineer crops sciences (2007), Faculty of Agronomy, University of Lubumbashi; Training in arbuscular mycorrhiza fungi (2016 and 2018) at department plants and crops, Faculty of Bioscienc Engineering, Ghent University, Gent, Belgium.