



International Journal of **Soil Science**

ISSN 1816-4978



Academic
Journals Inc.

www.academicjournals.com



Review Article

Production and Use of Arbuscular Mycorrhizal Fungi Inoculum in Sub-Saharan Africa: Challenges and Ways of Improving

¹R.W. Mukhongo, ¹J.B. Tumuhairwe, ^{1,2}P. Ebanyat, ³A.H. AbdelGadir, ⁴M. Thuita and ⁴C. Masso

¹School of Agricultural Sciences, Makerere University, Kampala, Uganda

²International Institute of Tropical Agriculture, Kampala, Uganda

³International Institute of Tropical Agriculture, Ibadan, Oyo State, Nigeria

⁴International Institute of Tropical Agriculture, Nairobi, Kenya

Abstract

Use of inorganic fertilizer is an essential practice to optimize crop productivity in the poor fertility soils in sub-Saharan Africa, but it has been linked to high cost of crop production, contamination of surface and/or ground water by nitrate leaching and eutrophication of surface water by phosphate run-off. Besides, secondary effects on soil biotic community and soil impoverishment have weakened cropping systems making them increasingly dependent on external chemical fertilizers. Efficient plant nutrition management should ensure both enhanced and sustainable agricultural production and safeguard the environment. Improved production and adoption of bio-inoculants such as arbuscular mycorrhizal fungi is an emerging soil fertility management practice with potential to increase and cheaply improve crop yields. Arbuscular mycorrhizal fungi inoculum production and adoption in sub-Saharan Africa smallholder systems is however, still limited mainly by research capacity and technological challenges. This study provides the state of the art in production and use of the technology and highlights the challenges and opportunities for its advancement. To experience the benefits of arbuscular mycorrhizal fungi, sound investment on research in low input systems and technical support from the government, the public and the private sectors should be considered. Nevertheless, adequate training of extension workers, agro-dealers and smallholder farmers through agricultural, academic and research institutions will solve the challenges of production and adoption of arbuscular mycorrhizal fungi inoculum technology hence improve crop production.

Key words: Arbuscular mycorrhizal fungi, production, challenges, way-forward, inorganic fertilizer

Received: January 28, 2016

Accepted: May 03, 2016

Published: June 15, 2016

Citation: R.W. Mukhongo, J.B. Tumuhairwe, P. Ebanyat, A.H. AbdelGadir, M. Thuita and C. Masso, 2016. Production and use of arbuscular mycorrhizal fungi inoculum in sub-Saharan Africa: Challenges and ways of improving. *Int. J. Soil Sci.*, 11: 108-122.

Corresponding Author: R.W. Mukhongo, School of Agricultural Sciences, Makerere University, Kampala, Uganda

Copyright: © 2016 R.W. Mukhongo *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Agricultural productivity in Sub-Saharan Africa (SSA) is gradually declining and attributed to increasing water stress, low soil fertility, especially nitrogen, phosphorus, potassium and pest and diseases. Great research and development effort has targeted plant breeding for high yielding combined with drought tolerance, pests and diseases resistance for several cereal, legume and root tuber crops¹. Measures and practices targeting soil have been low and yet constrained by poverty and climatic conditions that are ever changing^{2,3}. For instance, there is rapid decomposition of organic matter reducing the quantity of soil organic matter. Secondly, tropical soils in SSA are old and highly weathered, deficient of primary minerals, which would be source of plant nutrients. Consequently, N and P are subjected to leaching, while P is fixed by the secondary minerals. The practice of continuous cropping without soil input has exacerbated declining soil fertility⁴.

Improved fallows were adopted in SSA for rapid replenishment of soil fertility⁵ but are no longer feasible in many arable areas due to increased population pressure. The increased fertilizer costs and environmental degradation linked to the continuous use of inorganic inputs (water pollution from nitrates and phosphates) is increasingly expanding and sometimes irreversible⁶ particularly in developed countries. In some cases, secondary effects on soil biotic community and soil impoverishment have weakened cropping systems making them increasingly dependent on external inorganic inputs⁷. Demand for clean agriculture (products with minimum allowable residual toxic levels are required in the market), high-quality food and clear labeling information on food ingredients and how food is produced are finally having an effect on decreasing the level of inorganic inputs used in developed countries⁸. However, in developing countries the great need for food due to population pressure implies a trend toward intensification and sustainable agriculture⁹, mainly through the use of more inorganic fertilizers. Recently, the use of bio-inoculants such as rhizobial and Arbuscular Mycorrhizal Fungi (AMF) are emerging soil fertility management practical technologies with potential to cheaply improve crop yields yet environmental-friendly option to complement reduced rates of inorganic fertilizers¹⁰. Several studies have reported increased crop yield following application of rhizobial inoculants^{11,12} and AMF¹³⁻¹⁵. Bio-inoculants are products containing living cells of different types of microorganisms with ability to mobilize nutrients for plant use through biological process¹⁶.

Berg¹⁷ indicated that the global market for bio-inoculants is growing at an estimated rate of about 10% per annum; valued at \$440 million in 2012 and expected to reach \$1,295 million by 2020¹⁸. The market study indicated that rhizobia inoculants were the mostly used in 2012, constituting 79% of the world's demand followed by phosphate mobilizing bio-inoculants (15%) and others such as mycorrhizal inoculants (7%). However, demand is mainly driven from Asia, where governments, such as China and India are promoting the use of bio-inoculants through tax incentives, tax exemptions and grants to provide support for their manufacture and distribution. Smallholder farmers in SSA are barely using bio-inoculants¹⁹. The objective of this study is to explore methods of AMF inoculum production in SSA, the challenges and quality improvement in order to exploit the opportunities for scaling up and out the technology adoption in SSA.

PRODUCTION OF AMF INOCULUM

Vostáka *et al.*²⁰ reported that there are about 12 mycorrhizal inocula producers in the European Union, with the producers in the United Kingdom, Czech Republic, Germany, Switzerland, Spain and France and more than 20 others worldwide. Table 1 gives a worldwide list of some of the bio-inoculants containing mycorrhizal propagules and their manufacturers. From the list, one can deduce the low mycorrhizal inoculum production in SSA (25%; Kenya and South Africa) compared to the other parts of the world (75%).

Large-scale multiplication of AMF aiming to produce mycorrhizal inoculant for field applications is generally carried out in substrate-based (nursery beds, pots, concrete tanks), substrate-free (i.e., aeroponic boxes) and *in vitro* systems²¹. Commercial inocula produced using these systems are available in several countries, especially in Asia and Europe. However, the costs associated with the technology of inoculum production, including establishment of single cultures of AMF species, shipping and handling and development of the carrier substrate are borne by farmers and nursery owners²² making the technology expensive. Culturing AMF is conventionally labor-intensive, requiring large-scale production of plants in pots or nursery beds, from which the AMF inoculum can be harvested²³. However, the *in vitro* cultivation system has gradually been developed and has become a valuable tool to mass-produce contaminant free-AMF under strictly controlled conditions²⁴ (Table 2).

Table 1: List of bio-inoculants, composition and their manufacturers

Bio-inoculant	Composition	Manufacturer	Reference
Rhizatech	<i>Glomus mosseae</i>	Dudutech, Naivasha, Kenya	Kundu ⁹²
Rhizatech	<i>Glomus etunicatum</i>	Dudutech, Naivasha, Kenya	
Rhizatech	<i>Glomus intraradices</i>	Dudutech, Naivasha, Kenya	
Rhizatech	<i>Glomus aggregatum</i> , <i>G. etunicatum</i> , <i>G. intraradices</i> , <i>G. mosseae</i>	Dudutech, Naivasha, Kenya	Kavoo-Mwangi <i>et al.</i> ⁹³ and Mukhongo <i>et al.</i> ⁹⁴
Symbion vam plus	<i>Glomus</i> spp., <i>Gigaspora</i> spp., <i>Bacillus megaterium</i> var. <i>phosphaticum</i>	T. Stanes and Company Ltd., Peninsular, India	
Symbion vam	<i>Glomus fasciculatum</i> , other endomycorrhizal spp.	T. Stanes and Company Ltd., Peninsular, India	
Glomygel® Hortalizas	<i>Rhizophagus irregularis</i>	Mycovitro, Spain	
Mycormax	<i>Glomus</i> (2 spp.), ectomycorrhizal (ECM) (5 spp)	JH Biotech Inc., Ventera, US	Wiseman <i>et al.</i> ⁹⁵
BEI	<i>Glomus</i> (6 spp.), <i>Gigaspora</i> (1 sp.), <i>Paraglomus</i> (1 sp.)	Bio Organics Santa Maria, US	
AgBio endos	<i>Glomus</i> (6 spp.), <i>Gigaspora</i> spp.	AgBio Inc. WestMinister, US	
AM 120	<i>Glomus</i> (3 spp.)	Reforestation Technologies Int. Salinas, UK	
BioGrow endo	<i>Glomus</i> (3 spp.), <i>Trichoderma</i> spp.,	Mycorrhizal Applications Inc., Grants, US	
Die hard endo starter	<i>Glomus</i> (6 spp.), <i>Gigaspora</i> (9 spp.), <i>Trichoderma</i> (1 sp.)	Horticultural Alliance Inc., Sarasota, US	
Mycotree root dip	Ecto/Endomycorrhizae (5 spp.)	Plant Health Care Inc.	
Root dip universal	AMF spp., Beneficial bacteria	Tree Pro West Lafayette, in US	
Mycoroot supreme	Not specified	Mycoroot (Pty) Ltd., South Africa	
Mycosuper booster	Not specified	Mycoroot (Pty) Ltd., South Africa	
Mycoroot green	Not specified	Mycoroot (Pty) Ltd., South Africa	
Mycoroot supergrow	Not specified	Mycoroot (Pty) Ltd., South Africa	
MYKE PRO SG2	<i>G. intraradices</i>	Premier Tech Biotechnologies, Canada	Antunes <i>et al.</i> ⁹⁶
Earth roots	AMF spp.	Not included	Corkidi <i>et al.</i> ⁹⁷
MycosApply endo	<i>G. intraradices</i>	Not included	
VAM 80	Not specified	Not included	
Ascend PB	Not specified	Not included	
NTC	Not specified	Not included	
Symbivit	<i>Glomus</i> spp.	Symbiom Ltd., Czech Republic	
Ectovit	Ectomycorrhiza	Symbiom Ltd., Czech Republic	
Rhodovit	Not specified	Symbiom Ltd., Czech Republic	
Turfcomp	Not specified	Symbiom Ltd., Czech Republic	
Endorize	Not specified	Biorize	
Mycogro Ag®	Not specified	Fungi Perfecti®, Olympia, WA, New Zealand	Monk <i>et al.</i> ⁹⁸
Mycogro Hort®	Not specified	Fungi Perfecti®, Olympia, WA, New Zealand	
MycosApply endo	AMF (3 spp.)	Mycorrhizal Applications Inc., Grants, US	
MycosApply Micronized endo	AMF (3 spp.)	Mycorrhizal Applications Inc., Grants, US	
MycosApply root dip gel	AMF (12 spp.)	Mycorrhizal Applications Inc., Grants, US	
MycosApply endonet	<i>G. intraradices</i>	Mycorrhizal Applications Inc., Grants, US	
MycosApply soluble	7 AMF spp. (<i>G. intraradices</i> , <i>G. mosseae</i> , <i>G. aggregatum</i> , <i>G. clarum</i> , <i>G. deserticola</i> , <i>G. etunicatum</i> , <i>Gi. margarita</i>), 9 ECM spp. (<i>R. villosullus</i> , <i>R. luteolus</i> , <i>R. amylopogon</i> , <i>R. fulvigleba</i> , <i>P. tinctorius</i> , <i>Laccaria</i> (2 spp.), <i>Suillus</i> (2 spp.)), 2 <i>Trichoderma</i> spp. (<i>T. harzianum</i> , <i>T. konigii</i>), 19 bacterial spp. (<i>B. subtilis</i> , <i>B. licheniformis</i> , <i>B. azotoformans</i> , <i>B. megaterium</i> , <i>B. coagulans</i> , <i>B. pumilis</i> , <i>B. thuringiensis</i> , <i>B. stercoraria</i> , <i>P. polymyxa</i> , <i>P. durum</i> , <i>P. floescence</i> , <i>P. gordonae</i> , <i>A. polymyxa</i> , <i>A. chroococcum</i> , <i>S. cervisiae</i> , <i>Steromyces griseus</i> , <i>S. lydicus</i> , <i>P. aureofaceans</i> , <i>D. erythromyxa</i>)	Mycorrhizal Applications Inc., Grants, US	

The different methods of AMF inoculum production have associated advantages and disadvantages (Table 3). All the methods of AMF inoculum production in Table 2 are recommended for SSA though their use is dependent on the availability of resources. The use of conventional methods i.e., nursery beds, pot and concrete tank cultivation is feasible due to their reasonable costs of installation and maintenance. However, a lot of care is required to control cross-contamination of inoculum, which hinders wide application in SSA. Aeroponic box and *in vitro* cultivation

systems are quite costly to install and maintain hence, making them expensive for most smallholder farmers in SSA to make use of them. In case of availability of finances and the technical know-how, inoculum production companies and farmers would go for the aeroponic box and *in vitro* cultivation methods since they require limited time to culture pure spores that are contaminant-free. Incidentally, these technical and financial constraints constitute major challenges for SSA as will be explained later.

Table 2: Methods of arbuscular mycorrhizal fungi inoculum production

Method of production	Duration for production	Yield of propagules	Reference
Nursery bed cultivation: About 25 m ² plots are tilled and fumigated or solarized for 2-4 week, AMF are then inoculated into holes drilled in the soil and then seeds of a host plant e.g., <i>Bracharia decumbens</i> are sown or pre-colonized host plant is transplanted to the plot, minimizing the amount of starter inoculum needed. Flowers are removed during growth to avoid seeds falling to the soil and becoming a weed problem when the inoculum is used	Soil and roots are harvested to a depth of 20 cm after 4 months of growth	Spores of the introduced isolates in fumigated plots increase relative to indigenous AM fungi compared to un-fumigated, inoculated plots	Ijdo <i>et al.</i> ²¹
Nursery bed cultivation: Raised beds of soil (60×60×16 cm) are prepared and fumigated, AMF from pot culture (introduced or indigenous isolates) are inoculated into furrows in the beds as starter inoculum. A succession of hosts is grown over the course of 3 years e.g., <i>Sorghum sudanese</i> , <i>Zea mays</i> and <i>Daucus carota</i> may be grown in one year, each for 4 months. There is an economic return from each host crop	After the third cycle the soil in the raised beds is ready to be used as inocula	Amount of inoculum increases approximately 10-fold from year 1 to year 3, yielding upwards of 2.5 × 10 ⁶ propagules per bed	Smith <i>et al.</i> ⁵³ and Sieverding ⁹⁹
Nursery bed cultivation: Raised beds are prepared as in Gaur ¹⁰³ and Douds <i>et al.</i> ⁶⁷ using a 2:1 (vol/vol) mixture of soil to leaf compost and inoculated or left un-inoculated to increase indigenous AMF. Forage crops or vegetables are grown as host plants, giving an economic return in addition to AMF inoculum	Only 1 plant growth cycle is used	Inoculum production is 15-20 fold greater when starter inoculum was used, however, the method produced only 55-69,000 propagules per bed, 40-fold fewer than the 3-year cycles	Gaur and Adholeya ⁷⁹ and Sieverding ¹⁰⁰
Nursery bed cultivation in temperate climates: Raised bed enclosures, (0.75×3.25×0.3 m) are constructed with silt fence walls, weed barrier cloth floors and plastic sheeting dividing walls between 0.75 m ² sections. Enclosures are filled to a depth of 20 cm with mixtures of compost and vermiculite, an optimal 1:4 (vol/vol) mixture of compost and vermiculite, respectively. Host plants e.g. <i>Paspalum notatum</i> Flügge, pre-colonized by AMF are transplanted into the enclosures, one isolate per enclosure section. Enclosures are then tended for one growing season; watered as needed and weeded as seeds in the compost germinate. The host plant, being a tropical C4 grass is frost-killed naturally so as not to become a weed pest itself	Inoculum is produced over winters <i>in situ</i> and is ready for use the following growing season	Experimentation has shown that no-supplemental nutrient addition is necessary because of adequate spores	Bendavid-Val <i>et al.</i> ¹⁰¹
Pot culture cultivation: Two-thirds of clean pots are filled with sterilised soil, 20 g of AMF starter culture are added and sown with 10-15 sorghum seeds. The culture is grown for 3-4 months, cores of roots and soil are removed from each pot to check for inoculum quality (presence of mites, nematodes and contaminant fungi). Watering is reduced for 1 month pots are allowed to dry and shoots are removed. Cores are removed again to check for inoculum quality. Roots are chopped up and mixed with soil to standardize inoculum	6 months	Improved quality propagules but yield may be lower than for nursery bed production but it depends on the quantity of the culturing media	Schreiner <i>et al.</i> ¹⁰²
Concrete tank cultivation: A tank (1×1×0.3 m) is constructed and lined with black polythene, mixed 50 kg of vermiculite and 5 kg of sterilized soil are added to the tank up to a depth of 20 cm, 1 kg of AMF inoculum is spread 2-5 cm below the surface of vermiculite-soil mixture, surface sterilized seeds of a host plant e.g., maize are sown, some N, P and K is applied at sowing and N at topdressing depending on nutrient levels of the culturing media, on the 30th and 45th day of sowing AMF root colonisation is analysed, stock plants are grown for 60 days, roots of the host plants are cut and mixed with the culturing media to obtain inoculum	2 months	Improved quality propagules but yield may be lower than that of nursery beds depending on the size of the tank	Gaur ¹⁰³
Aeroponic box cultivation: Pre-colonized plants are suspended in a chamber, in which a mist of nutrient solution is generated from an atomizing disk or pressurized spray, when all goes well, roots are amply colonized within 90 days, at harvest roots are removed, washed over a coarse sieve to remove and separate spores. The clean root fragments are sheared further in a food processor. This material is collected on a fine sieve and used as "sheared-root" inoculum	3 months	High quality propagules greater in than those produced in soil-based media	Gaur ¹⁰³
In vitro cultivation: Potential viable mycorrhizal propagules are extracted from soil, surface sterilized and growth conditions are optimized for aseptic germination. The association of propagules with a suitable excised root and recovery of the produced propagules then follows. Mass-produced propagules are then formulated in utilizable formulations such as wettable powders, granules or liquid suspensions for application on target crops	Short time	Quality and yield of propagules is greater than that of other production methods	Adholeya <i>et al.</i> ⁷⁷ and Douds <i>et al.</i> ⁵⁷

Table 3: Advantages and disadvantages of various arbuscular mycorrhizal fungi inoculum production methods

Factor	Nursery bed cultivation			Pot/Concrete tank cultivation			Aerobic box cultivation			In vitro cultivation		
	Advantage	Disadvantage	Advantage	Disadvantage	Advantage	Disadvantage	Advantage	Disadvantage	Advantage	Disadvantage	References	
Cost of installation/production	Adapted for local use, low costs	Not well adapted for industrial development	Adopted for local use, reasonable costs	Limited in their industrial development	Can be adopted for industrial development	High cost	Can be adopted for industrial development	High cost	Can be adopted for industrial development	High cost	Gaur <i>et al.</i> ⁹⁴	
Life cycle completion	Suitable for most strains	Time and space consuming, destructive sampling	Suitable for most strains	Time and space consuming, destructive sampling	Short life cycle strains	Few spores produced	Short life cycle destructive sampling	Few spores produced	Short life cycle destructive sampling	Few spores produced	Douds <i>et al.</i> ⁹⁵	
Sub-culturing	Suitable for most strains	Time and space consuming, destructive sampling	Suitable for most strains	Time and space consuming, destructive sampling	Easy extraction of propagules	Each fungus accession needs a chamber	Time and space saving, non-destructive observation	Some strains resistant to sub-culturing	Time and space saving, non-destructive observation	Some strains resistant to sub-culturing	Ingleby ⁹⁶	
Consistency in quality	Consistency not guaranteed	Rarely consistent	Somehow consistent	Contamination fairly eliminated	Consistent results	Investigated for few strains	Consistent results	Investigated for few strains	Consistent results	Investigated for few strains	Gianinazzi and Vosatka ²³	
Viability germination potential	Numerous strains maintained	Substrate is sampled	Numerous strains maintained	Substrate is sampled	Viability assessment is easy	Lack of culturing media affect spore production	Viability assessment is easy	Low sporulation levels of some strains	Viability assessment is easy	Low sporulation levels of some strains	Jarstfer and Sylvia ⁹⁷	
Stability	Easy to maintain	Space and time consuming	Easy to maintain	Space and time consuming	Some known culturable species have been tested	Limited species show compatibility	Non-changing growth conditions throughout generation	Sub-cultivation may decrease infectivity and effectiveness	Non-changing growth conditions throughout generation	Sub-cultivation may decrease infectivity and effectiveness	Raman <i>et al.</i> ⁹⁸	
Purity	Purity not guaranteed	Rarely pure	Purity not guaranteed	Contamination fairly eliminated	Contamination greatly eliminated	Algae grow in nutrient solution	Pure spores	Investigated for few strains	Pure spores	Investigated for few strains	Adholeya <i>et al.</i> ⁷⁷	
Identity	Classical tools and literature are compared	Limited descriptive tools	Classical tools and literature are compared	Limited descriptive tools	Multidisciplinary approach	No disadvantage	Multidisciplinary approach	No disadvantage	Multidisciplinary approach	No disadvantage	Fortin <i>et al.</i> ⁵⁸	
Volume of inoculum	Large spore quantities produced	High cost of transport due to large volume	Large spore quantities produced	Slightly high cost of transport due to large volume	Small volume hence low cost of transport	Few spores produced	Small volume hence low cost of transport	Low sporulation for some strains	Small volume hence low cost of transport	Low sporulation for some strains	IAEA ⁴⁶	
Long-term preservation	Demonstrated for various species	Contamination is common	Demonstrated for various species	Slight contamination is common	Less space required	Storing sheared roots is hard	Long-term preservation is feasible	Preservation only tested for few species	Long-term preservation is feasible	Preservation only tested for few species	Declerck <i>et al.</i> ⁹⁹	

OPPORTUNITIES FOR ADOPTING AMF IN SSA

The major constraints to agricultural production in SSA were highlighted in the introduction as water stress, low soil fertility, pests and diseases. Low soil fertility is manifested by the increasing nutrient balances²⁵. Secondly, tropical soils are highly weathered, acidic and characterized by P fixation. The AMF improve soil structure through particle binding²⁶ and can hence be applied to improve physically degraded soil, which is prevalent in the majority of subsistence farmers. Mycorrhiza symbiotic relationships are known to improve moisture and P uptake by plants. These mechanisms can be exploited to improve plant survival under drought condition and release of sesquioxide fixed P within the soil. There are widespread opportunities for application of AMF in SSA at both subsistence and commercial farming systems. Other reported benefits of AMF may involve crop protection against phytopathogens²⁷⁻²⁹. At plant community level AMF reduce competition for water and nutrient thus influencing plant biodiversity³⁰ and sustainability of terrestrial ecosystems. The AMF symbiosis can mitigate the negative effects of water stress on plant growth^{31,32} although, the effects are often subtle, transient and probably circumstance and symbiont-specific³³. Commonly observed benefits of AMF are improved uptake of nutrients especially P, but also ammonium (NH₄⁺), calcium (Ca), sulfur (S), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu)³⁴⁻³⁶. Some developed countries in the world have experienced greater development in production and use of AMF³⁷, while farmers in SSA are not benefiting from it since the production and awareness on the benefits is low, therefore, they lack knowledge and understanding of the technology. The production of AMF inoculum in SSA faces several challenges which negatively impact the adoption of the technology.

CHALLENGES TOWARDS PRODUCTION AND ADOPTION OF AMF INOCULANTS IN SSA

The AMF production and adoption in SSA smallholder systems is still limited due to lack of awareness and understanding of bio-inoculum²¹ hence, poor development of the sector³⁸ despite numerous reports on the central role of bio-inoculum in sustainable agriculture. Understanding how these challenges affect the production and adoption of bio-inoculants may improve the benefits of AMF inoculum among smallholder farmers in SSA. Research capacity and technological challenges are the main hindrance to AMF inoculum production and adoption.

RESEARCH CAPACITY CHALLENGES

Lack of suitable facilities for AMF inoculum production and storage: The SSA population in 2014 was estimated at 973.4 million people³⁹, of which over 60% is involved in agriculture. Secondly, there are about 0.23 ha of arable land per person under agriculture constituting about 14.8% of arable land. The rate of inorganic fertilizer application is at an average of 13.22 kg ha⁻¹⁴⁰. Efforts to reach at least 50 kg of fertilizer per hectare by the Abuja declaration of 2006 have had less success due to high cost of fertilizer and low purchasing power of farmers⁴¹⁻⁴³. Rhizobial bio-inoculants that are commonly used in SSA have demonstrated to economically increase yields in many part of the region¹⁹. This has been supported by the fact that many of SSA countries have units that produce the rhizobial inoculants because of availability of human and infrastructural capacity hence, low inoculant cost. For example, in Kenya MEA Ltd., in collaboration with the University of Nairobi produces inoculants for beans, soybean and groundnut⁴⁴ and Madhavani Ltd and Makerere University in Uganda also produce rhizobial inoculants for common grain legumes. Similarly, Rwanda Agricultural Board and Sokoine University of Agriculture had rhizobial production units in Rwanda and Tanzania, respectively¹⁹. In West Africa, the Microbiological Resource Centre (MIRCEN) in Senegal (Dakar) produces inoculant for cowpea, groundnut, soybean, common beans, acacia and sesbania species and the French Institute of Scientific Research for Cooperative Development (ORSTOM) in Dakar that is involved in research activities focusing on legume-rhizobium symbioses⁴⁵. In Southern Africa, Chitedze Agricultural Research Station, Lilongwe in Malawi started producing commercial inoculants for soybean and cowpea in 1970s⁴⁶. Zimbabwe has a large and well-established commercial BNF technology soybean sector⁴⁷ spearheaded by the Soil Productivity Research Laboratory (SPRL) and supported by the International Atomic Energy Agency⁴⁸. On the contrary, mycorrhizal inoculants are less available to farmers in SSA because there are few AMF inoculant production units in the region, only known to exist in Kenya by Dudutech and Mycoroot Pty Ltd in South Africa. In case of increased demand for the inoculum by smallholder farmers or large-scale farms in Kenya, Dudutech Ltd may not produce enough for the clients. This confirms the fact that most of the AMF inoculum used in SSA is imported hence, high market prices due to production and transportation costs. Due to high prices of the imported inoculants, on-farm production of AMF inoculum is considered an attractive alternative⁴⁹ to inoculant importation.

Culturing AMF conventionally is labor-intensive, requiring large-scale production of plants in pots or nursery beds from which the AMF inoculum can be harvested²³. Moreover, the bulkiness of the carrier material of conventionally produced inoculum makes its use less feasible^{50,51}. Owing to the obligate bio-trophic nature of AMF, their infective propagules are produced and preserved in small nursery beds (~1 m²) using mycotrophic crops or in continuous pot cultures. Producing and maintaining monospore cultures in nursery beds is also a challenge due to high risk of contamination by indigenous microorganisms of no interest. To overcome the challenge of contamination, it may require methods that use sterile culturing media i.e., pot cultures, concrete tank cultures and aeroponic box cultures. Moreover, these methods have an advantage over use of nursery beds in terms of reduced inoculum volume/bulkiness hence, increased feasibility in application. Nonetheless, the on-farm production of inoculum avoids some of production and transportation costs and the technology can be easily transferred to farmers²² and it may somehow solve the problem of high market inoculum prices, low quality and poor delivery mechanisms associated with production and storage conditions when compared with the imported inoculum. On-farm production of inoculum from locally isolated adapted species may be more effective than introduced ones in certain situations⁵². Furthermore, a taxonomically functional diverse inoculum can be produced^{53,54} as opposed to commercial inocula, which may contain only one species⁴⁹. A formulation containing a consortium of AMF strains would have several advantages over single-isolate AM fungal inocula³⁷, since a single strain may not be able to withstand certain environmental changes. Academic institutions in SSA multiply AMF spores from local soils to culture single and/or composite strain(s) inocula that are compatible with local environmental conditions, hence, can successfully compete with native ones^{55,56}.

A sustainable solution to the quality and affordability of inoculum challenge may be the use of *in vitro* cultivation system^{57,58}. This technology can be adopted if the national governments in SSA can install *in vitro* cultivation systems in their decentralized research organizations and procure qualified personnel to train agricultural extension officers and farmers on inoculum production. It is important that academic, governmental and industrial scientists in SSA collaborate jointly to improve their knowledge on the *in vitro* technology and develop its use, with efforts to release quality products to the market. The technology has so far been transferred to two leading agriculturally and pharmacologically based industries in India³⁷. *Rhizophagus irregularis* formerly *Glomus intraradices* has been produced

in an artificial *in vitro* AMF culture system with *Agrobacterium rhizogenes*-transformed carrot roots by Mycovitro in Spain.

Storage of bio-inoculants requires special facilities and skills, which most producers, agro-dealers and farmers do not possess. Storing bio-inoculants under non-refrigerated conditions may lead to loss of viability of the microbial cells/propagules. The inoculum producing companies should target seasons for high inoculum demand to overcome the challenge of proper storage facilities. A standard cold room will require intervention of national governments to subsidize the costs and make the inoculant products affordable to the farmers. In places where electricity supply exists, companies should invest in modern cold rooms, if not so, then in traditional cold stores for the storage of inoculum.

Lack of qualified personnel: There is a scarcity of trained human resource in AMF technology in SSA. Therefore, the production units may lack qualified personnel which in turn affect the quality of bio-inoculants. The production staff should be equipped with knowledge and skills on isolation, identification, examination and selection of improved strains having greater crop diversification and survival during transport, storage and after soil application. It is also important to have ecological knowledge affecting the fungi such as pH, nutrient deficiencies, salinity, high temperature and presence of toxic elements on survival and establishment of inoculum and efficacy of bio-inoculants in varying regions⁵⁹. These ecological constraints are widespread in SSA. It is important to tailor effective bio-inoculants for specific regions. Lack of qualified personnel is a challenge that leads to lack of awareness and understanding of bio-inoculum technology²¹, which has also negatively impacted development of the AMF production industry³⁸. Study organizations and bio-inoculum producing companies in SSA can improve the key stakeholders through participatory demonstration trials who in-turn can train farmers in their communities. Smallholder farmers, agro-dealers, extension service workers and policy makers should be trained through a participatory approach on the beneficial aspects of AMF, selection and preservation of effective species for production and wide adoption of inoculum^{60,61}.

TECHNOLOGICAL CHALLENGES

Formulation carrier materials: Formulation technologies largely take care of possible adverse environmental effects and factors that may render the inoculum ineffective and it may be a challenge to its commercialization⁶². Formulation is a blend of microbial propagules with carrier materials into a

Table 4: Advantages and disadvantages of commonly used arbuscular mycorrhizal fungi formulation carrier materials

Carrier	Advantages	Disadvantages	Reference
Peat	Suitable for a wide range of microorganisms: Bacteria, AMF, ECM, protective nutritive environment, moisture content can be adjusted to optimize growth and survival during curing, storage and transport	Not readily available, strong negative impact on the environment and the ecosystems, extraction is costly, toxic compounds released during drying and sterilization, highly variable in composition and quality depending on the origin, holds a load of microorganisms which can reduce the shelf life of the inoculant	Malusa <i>et al.</i> ⁶⁸
Granules	Easy to store handle and apply, less dusty than peat, application rate easily assessed, no toxicity during soil application since there is no direct contact with other chemical compounds, more efficient under stressful environmental conditions	Bulky: high transport and storage costs, higher application rates, often non-sterile	Chabot <i>et al.</i> ¹¹⁰
Compost	Pure cellulose from well composted materials can increase asymbiotic hyphal growth of AMF	Cellulose in compost materials that are not well composted can reduce the mycorrhization rate	Declerck <i>et al.</i> ¹¹¹
Coal, clays and inorganic soils	Available in different regions	Their microbial load depends on the site of production (about 10 ² -10 ³ CFU g ⁻¹), but it is generally lower than in organic carriers	Herrmann and Lesueur ¹¹²

product that can be effectively delivered and applied to the target crop. Carrier substrates should be well selected to provide a stable environment for microbial fractions, prolong inoculum shelf-life and act as dispersal and dissolution vectors in soil. A successful formulation carrier must be economically viable to produce, with no deleterious effects on the mycorrhizal symbiosis, easy to handle during transportation and application and allow effective dispersion near the roots⁴⁹. It should also possess the following properties: Good moisture retention capacity, easy to process and free of lump-forming materials, near-sterile or easy to sterilize by autoclaving or by other methods (e.g., gamma-irradiation) and good pH buffering capacity⁶³, a standardized composition ensuring chemical and physical stability, suitability for as many plant growth promoting microorganism species and strains as possible, the possibility of mixing with other compounds (nutrients or adjuvants) and being composed of biodegradable and non-polluting compounds⁶⁴. The AMF inoculum must be formulated in such a way that they can be stored and distributed under a wide range of temperatures without losing viability²³.

Unavailability of good quality carrier material or use of different carrier materials by producers without establishing the quality of the materials is a hurdle in bio-inoculum production. It is also difficult to ensure consistency in AMF inoculum quality because of the carrier material used in the conventional production methods²³. Since in these methods the culturing media is used as the carrier material, the quality of the media or carrier material should be determined prior to inoculum production or packaging. Quality control of the culturing media or carrier material should include determining their microbial composition, which is eliminated through

sterilization and their physical and chemical composition, which should be adjusted to the optimum levels for the increased viability of the inoculum depending on AMF species. Several mycorrhizal inoculum formulations have been used: glass beads at the research laboratory level⁶⁵, expanded clay in the commercial sector⁶⁶, inert carriers such as sand, vermiculite, perlite and soil-rite (soilless compost)^{67,68}, powder, tablets/pellets or granules, gel beads and balls³⁷, alginate beads⁶⁹, soil materials (clay, coal and peat) and organic materials (compost)⁶⁸. The formulation carrier materials have advantages and disadvantages (Table 4) and the disadvantages constraint bio-inoculants production and their subsequent performance in the field.

Shelf-life of inoculum: One of the major challenges faced by the producers of bio-inoculants and investors is inadequate demand and the inconsistent and seasonal nature of the existing demand, necessitating efficient storage⁵⁹. Besides, most national standards regulatory bodies may lack capacity to check the quality of AMF inoculants. Shelf-life is determined by the production technology, carrier and packaging material used, mode and distance of transport and storage. Most bio-inoculants in the market in SSA are imported and generally not tailored to the local conditions in terms of shelf-life and storage conditions especially by smallholder farmers⁷⁰ and agro-dealers. It is thus important for large-scale and on-farm inoculum producers to carry out quality control analysis on formulated bio-inoculants^{71,72} in various storage conditions and periods to ensure product viability over a significant period of time. They should consider their shelf-life, date of manufacture and date of expiry.

INCONSISTENT PERFORMANCE

Inconsistent field performance is the major constraint associated with marketing of bio-inoculants because it raises concerns about sustainable benefits of the inoculants⁵⁹. While culturing AMF strains for inoculum production, the environmental conditions of the origin of strains and where the inoculum is to be used should be considered⁵⁷. This is important for adaptability of AMF in the different local SSA edaphic and climatic conditions. The physiological characteristics of the inoculant microorganism determine to a greater extent its survival and activity in soil. Hence, different species will show varying responses, in terms of survival and activity. Packaging of improper or less efficient strains for production could be another challenge facing AMF inoculum production in SSA. The correct isolation, identification and examination of the potential roles of AMF in SSA region could be imperative. With thorough screening, potentially infective and effective AMF for the region could be identified and supplied to smallholders in the region for use. Ensuring consistency of product type and formulation appears challenging to the industry, even between supposedly similar products, i.e., different batches have varying quality. This can partially be achieved by including both spores and root fragments in the inoculum packages since spores persist longer within the soil environment but they are slow to colonise host plants compared to root fragments⁷³. Most importantly, evaluation of inoculum from commercial units with certain reference values to ensure the strict adherence to the protocols and methodologies recommended by recognized and independent laboratories is needed. This is most vital, as several handling errors occur at the industrial level during technology adoption and implementation, causing subsequent problems in product quality, which may lead to the dissatisfaction of both the end users and producers.

MARKET CHAIN

There are challenges of sustaining the quality of AMF inoculants from the production unit through input dealers to the farmers. A common practice in SSA is storage of products on shelves in agro-dealer's stores, where temperatures are usually quite high instead of being stored in refrigerated conditions since access to refrigerators or power is a great challenge. Besides poor storage conditions, unreliable agro-dealers can adulterate bio-inoculants along the commercialization chain, which requires periodic monitoring of products in the market to ensure product quality⁷⁴. It is also

necessary to consider the package sizes appropriate for the farmer. Re-packaging of bio-inoculants by agro-dealers into smaller packets for smallholder farmers in SSA may promote contamination hence, the product's poor quality. In SSA markets such as Kenya, Nigeria and Ethiopia⁷⁵, lack of continuous market monitoring has contributed to the presence of poor quality bio-inoculants and low demand of the inoculants by farmers, a situation expected in a majority of the SSA countries. It is prudent that individual countries set up regulations, regulatory body and functional independent laboratories with strengthened institutional capacity to monitor the quality of bio-inoculants⁷⁶. This will help to maintain quality and effective bio-inoculants on the market, gain end-users trust and eventually boost production due to increased demand.

CULTURING MEDIA

The choice of culturing media used has also been shown to affect inoculant success⁷⁷. For instance, application of lime lowers AMF root colonisation in field soils thereby reducing the dependence of the trap plants on mycorrhizae and restricted development of the fungi in root cortex. If sandy culturing media is available for spore multiplication, supplemental nutrients are required for increased AMF spore production⁷⁸, which is not the case for clayey media.

Organic matter is considered to encourage microbial activity, however, if cellulose is fresh or it is not well composted, it can inhibit AMF extraradical hyphae growth and root colonisation⁷⁹. Pure cellulose obtained after proper decomposition, increases AMF extraradical hyphae growth and root colonisation^{80,81}. These reports show the need of determining the level of decomposition of cellulose in inoculum culturing media. Therefore, the analysis of culturing media for their nutrient content is necessary but will lead to increased cost of inoculant production. Culturing media with high nutrient levels especially P may reduce plant dependency on AMF for nutrient uptake⁸², thereby reducing C allotted to AMF by the trap plant. This will eventually reduce the rate of colonisation of the trap plant hence, reduced rates of spore production. Readily available soil P and hence, increased plant P uptake may result in a shift in AMF community structure and reduced AMF diversity⁸³⁻⁸⁵. At higher plant tissue P concentration, plants tend to reduce root exudation that act as signal molecules for AMF spore germination and/or their hyphal branching⁸⁶ and allocate relatively more photosynthates to shoots and leaves instead of to the roots⁸⁷. Reduced exudation results in low AMF colonisation and spore production⁸⁸⁻⁸⁹. However, in nutrient-deficient culturing media,

Table 5: Effect of soil phosphorus levels on functioning of arbuscular mycorrhizal fungi

Phosphorus in/added to P-deficient soils	State of AMF colonisation	References
Soils with 220 mg P kg ⁻¹	Mycorrhizal infections tend to die out	Smith ¹¹³
1.5 g P kg ⁻¹ or more of mono-calcium phosphate	Mycorrhizal infection virtually disappeared inhibiting colonisation by mycorrhizae	Baylis ¹¹⁴ and Mosse <i>et al.</i> ¹¹⁵
Soils given 280 µg P g ⁻¹	Decreased colonised root length and intensity of AM fungal biomass per colonised root length	Menge <i>et al.</i> ¹¹⁶ and Jasper <i>et al.</i> ¹¹⁷
At 50 mg P kg ⁻¹	Greatest mycorrhizal symbiosis	Thompson ¹¹⁸
At 100 mg P kg ⁻¹	No mycorrhizae effectiveness	Schubert and Hayman ¹¹⁹
Bicarbonate-soluble p>140 mg kg ⁻¹	Decreased rate of colonisation	Amijee <i>et al.</i> ¹²⁰
Soils with >25 mg P kg ⁻¹	Greatest benefits of AMF	Sastry <i>et al.</i> ¹²¹

addition of P fertilizer may not be sufficient to reduce root exudation, therefore, AMF diversity and colonisation may be stimulated^{90,91}. Phosphorus management after analysis of culturing media is essential for optimal functioning of AMF plant symbiosis⁸⁴, which greatly influences AMF sporulation (Table 5).

CONCLUSION

Despite numerous reports on the central role of AMF in sustainable agriculture, most smallholder farmers in SSA are not aware of AMF benefits and do not have access to AMF inoculum and hence, they experience low crop yield. Isolation, identification and examination of the potential of local AMF strains in SSA should be considered and this will require deliberate investment in research of low input systems and technical support from the government, the public and the private institutions. This coupled with adequate training of extension workers and smallholder farmers through agricultural, academic and research institutions will help them learn how to optimize production and adoption of AMF inoculants to improve crop production.

ACKNOWLEDGMENT

The authors of this study would like to acknowledge the authors of the referenced articles.

SIGNIFICANCE STATEMENT

We believe these findings will be of great interest to policy makers, soil microbiology researchers, Arbuscular Mycorrhizal Fungi (AMF) inoculum producers, agricultural extension officers, agricultural companies and farmers who wish to use AMF inoculum. Arbuscular mycorrhizal fungi exist naturally and enter a symbiotic relationship with about 90% of terrestrial plants. Use of superior strains of AMF as inoculum can complement lower rates of inorganic phosphorus fertilizer. The adoption of the reviewed ways of improving the

production and use of AMF inoculum by the governments, research and academic institutions, extension officers and farmers will greatly contribute to increased crop production.

REFERENCES

1. Sleper, D.A. and J.M. Poehlman, 2006. Breeding Field Crops. 5th Edn., Wiley-Blackwell Publ., Nerw York, USA., ISBN-13: 9780813824284, Pages: 424.
2. Bekunda, M.A., A. Bationo and H. Ssali, 1997. Soil Fertility Management in Africa: A Review of Selected Research Trials. In: Replenishing Soil Fertility in Africa, Buresh, R.J., P.A. Sanchez and F.G. Calhoun (Eds.). Soil Science Society of America, Madison, USA., ISBN-13: 9780891188292, pp:63-79.
3. Sserunkuuma, D., J. Pender and E. Nkonya, 2001. Land management in Uganda: Characterization of problems and hypotheses about causes and strategies for improvement. Project on Policies for Improved Land Management in Uganda, International Food Policy Research Institute, March, 2001, Uganda.
4. Sanchez, P.A., 2002. Soil fertility and hunger in Africa. *Science*, 295: 2019-2020.
5. Sanchez, P.A., 1999. Improved fallows come of age in the tropics. *Agrofor. Syst.*, 47: 3-12.
6. Tilman, D., J. Fargione, B. Wolff, C. D'Antonio and A. Dobson *et al.*, 2001. Forecasting agriculturally driven global environmental change. *Science*, 292: 281-284.
7. Gyaneshwar, P., G.N. Kumar, L.J. Parekh and P.S. Poole, 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil*, 245: 83-93.
8. Boiffin, J., E. Malezieux and D. Picard, 2001. Cropping Systems for the Future. In: Crop Science: Progress and Prospects, Struik, P.C. J. Nosberger and H.H. Geiger (Eds.). CAB International, Oxford, UK., ISBN-13: 9780851998916, pp: 261-279.
9. Pretty, J., 2008. Agricultural sustainability: Concepts, principles and evidence. *Philos. Trans. R. Soc. B*, 363: 447-465.
10. Sharma, S.B., R.Z. Sayyed, M.H. Trivedi and T.A. Gobi, 2013. Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus, Vol. 2. 10.1186/2193-1801-2-587.

11. Tran, Y.T., 2004. Response to and benefits of rhizobial inoculation of soybean in the south of Vietnam. Proceedings of the 4th International Crop Science Congress, September 26-October 1, 2004, Brisbane, Australia.
12. Ndakidemi, P.A., F.D. Dakora, E.M. Nkonya, D. Ringo and H. Mansoor, 2006. Yield and economic benefits of common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) inoculation in Northern Tanzania. Aust. J. Exp. Agric., 46: 571-578.
13. Sumathi, C.S., V. Balasubramanian, N. Ramesh and V.R. Kannan, 2008. Influence of biotic and abiotic features on *Curcuma longa* L. plantation under tropical condition. Middle-East J. Sci. Res., 3: 171-178.
14. Ortas, I., 2010. Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions. Spanish J. Agric. Res., 8: S116-S122.
15. Ceballos, I., M. Ruiz, C. Fernandez, R. Pena, A. Rodriguez and I.R. Sanders, 2013. The *in vitro* mass-produced model mycorrhizal fungus, *Rhizophagus irregularis*, significantly increases yields of the globally important food security crop cassava. PLoS One, Vol. 8. 10.1371/journal.pone.0070633
16. Ahmad, F., S. Uddin, N. Ahmad and R. Islam, 2013. Phosphorus-microbes interaction on growth, yield and phosphorus-use efficiency of irrigated cotton. Arch. Agron. Soil Sci., 59: 341-351.
17. Berg, G., 2009. Plant-microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. Applied Microbiol. Biotechnol., 84: 11-18.
18. Transparency Market Research, 2014. Biofertilizers (biological nitrogen fixation, phosphate solubilisers and others) market for seed treatment and soil treatment applications. Global Industry Analysis, Size, Share, Growth, Trends and Forecast 2013-2019, Transparency Market Research, Albany, New York.
19. Chianu, J.N., E.M. Nkonya, F.S. Mairura, J.N. Chianu and F.K. Akinnifesi, 2011. Biological nitrogen fixation and socioeconomic factors for legume production in sub-Saharan Africa: A review. Agron. Sustain. Dev., 31: 139-154.
20. Vostáka, M., J. Albechtrova and R. Pattern, 2008. The International Market Development for Mycorrhizal Technology. In: Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics, Varma, A. (Ed.). Springer, New York, USA., ISBN: 9783540788263, pp: 419-438.
21. Ijdo, M., S. Cranenbrouck and S. Declerck, 2011. Methods for large-scale production of AM fungi: Past, present and future. Mycorrhiza, 21: 1-16.
22. Douds, Jr. D.D., G. Nagahashi, P.E. Pfeffer, C. Reider and W.M. Kayser, 2006. On-farm production of AM fungus inoculum in mixtures of compost and vermiculite. Bioresour. Technol., 97: 809-818.
23. Gianinazzi, S. and M. Vosatka, 2004. Inoculum of arbuscular mycorrhizal fungi for production systems: Science meets business. Can. J. Bot., 82: 1264-1271.
24. Declerck, S., D.G. Strullu and J.A. Fortin, 2005. *In Vitro* Culture of Mycorrhizas. Springer, New York, USA., ISBN: 9783540273318, Pages: 392.
25. Roy, R., R. Misra, P. Lesscheen and M. Smaling, 2003. Assessment of soil nutrient balance, approaches and methodologies. Fertilizer and Plant Nutrition Bulletin No. 14. FAO United Nations, Rome.
26. Rillig, M.C. and D.L. Mummey, 2006. Mycorrhizas and soil structure. New Phytol., 170: 41-53.
27. Elsen, A., S. Declerck and D. de Waele, 2001. Effects of *Glomus intraradices* on the reproduction of the burrowing nematode (*Radopholus similis*) in dioxenic culture. Mycorrhiza, 11: 49-51.
28. Forge, T., A. Muehlchen, C. Hackenberg, G. Neilsen and T. Vrain, 2001. Effects of preplant inoculation of apple (*Malus domestica* Borkh.) with arbuscular mycorrhizal fungi on population growth of the root-lesion nematode, *Pratylenchus penetrans*. Plant Soil, 236: 185-196.
29. Harrier, L.A. and C.A. Watson, 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 Manage. Sci., 60: 149-157.
30. Mummey, D.L., P.M. Antunes and M.C. Rillig, 2009. Arbuscular mycorrhizal fungi pre-inoculant identity determines community composition in roots. Soil Biol. Biochem., 41: 1173-1179.
31. Smith, S.E., E. Facelli, S. Pope and F.A. Smith, 2010. Plant performance in stressful environments: Interpreting new and established knowledge of the roles of arbuscular mycorrhizas. Plant Soil, 326: 3-20.
32. Jayne, B. and M. Quigley, 2014. Influence of arbuscular mycorrhiza on growth and reproductive response of plants under water deficit: A meta-analysis. Mycorrhiza, 24: 109-119.
33. Auge, R.M., 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza, 11: 3-42.
34. Harikumar, V.S. and V.P. Potty, 2007. Arbuscular mycorrhizal inoculation and phosphorus mobility in phosphorus-fixing sweet potato soils. Malaysian J. Soil Sci., 11: 45-56.
35. Van der Heijden, M.G.A., R. Streitwolf-Engel, R. Riedl, S. Siegrist and A. Neudecker *et al*, 2006. The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. New Phytol., 172: 739-752.
36. Hu, S. and T. Ruffy, 2007. Linking arbuscular mycorrhizal fungi with plant health: Mechanisms and challenges. Phytopathology, 97: 142-142.
37. Adholeya, A., P. Tiwari and R. Singh, 2005. Large Scale Inoculum Production of Arbuscular Mycorrhizal Fungi on Root Organs and Inoculation Strategies. In: *In vitro* Culture of Mycorrhizas, Declerck, S., D.G. Strullu and A. Fortin (Eds.). Springer, New York, USA., ISBN: 9783540273318, pp: 315-338.

38. Schulte-Geldermann, E., 2013. Tackling Low Potato Yields in Eastern Africa: An Overview of Constraints and Potential Strategies. In: Seed Potato Tuber Production and Dissemination, Woldegiorgis, G., S. Schulz and B. Berihun (Eds.). Ethiopia Institutes of Agricultural Research, Bahir Dar, Ethiopia, pp: 72-80.
39. World Bank, 2014. The World Bank annual report 2014. The World Bank, Washington, DC., USA.
40. World Bank, 2011. The World Bank annual report 2011. The World Bank, Washington, DC., USA.
41. Hassan, R.M., F. Muriithii and G. Kamau, 1998. Determinants of Fertilizer Use and the Gap Between Farmers Maize Yields and Potential Yields in Kenya. In: Maize Technology Development and Transfer: A GIS Application for Research Planning in Kenya, Hassan, R.M. (Ed.). CAB International, Wallingford, UK., ISBN-13: 9780851992877, pp: 137-168.
42. Achieng, J.O., D. Friesen, O. Odongo and M. Odendo, 2001. Sustainability of fertilizer use in maize production in Western Kenya through provision of credit. Proceedings of the African Crop Science Conference, Volume 5, October 21-26, 2001, Lagos, Nigeria, pp: 601-604.
43. Duflo, E., M. Kremer and J. Robinson, 2006. Understanding technology adoption: Fertilizer in Western Kenya evidence from field experiments. Preliminary and Incomplete, April, 2006, Western Kenya.
44. Odame, H., 1997. Biofertilizer in Kenya: Research, production and extension dilemmas. Biotechnol. Dev. Monitor, 30: 20-23.
45. Bala, A., N. Karanja, M. Murwira, L. Lwimbi, R. Abaidoo and K. Giller, 2011. Production and use of Rhizobial inoculants in Africa. Milestone Reference No. 3.4.1, January, 2011, South Africa, pp: 2-21.
46. Khonje, D.J., 1989. Adoption of the rhizobium inoculation technology for pasture improvement in sub-Saharan Africa. Department of Agricultural Research, Chitedze Agricultural Research Station, Lilongwe, Malawi, pp: 1-14.
47. Mpeperek, S., F. Javaheri, P. Davis and K.E. Giller, 2000. Soyabeans and sustainable agriculture: Promiscuous soyabeans in Southern Africa. Field Crops Res., 65: 137-149.
48. IAEA., 1998. IAEA annual report for 1998. GOV/1999/28, International Atomic Energy Agency, Austria, April 26, 1999.
49. Douds, Jr. D.D., G. Nagahashi, P.E. Pfeffer, W.M. Kayser and C. Reider, 2005. On-farm production and utilization of arbuscular mycorrhizal fungus inoculum. Can. J. Plant Sci., 85: 15-21.
50. Pellegrino, E., A. Turrini, H.A. Gamper, G. Cafa, E. Bonari, J.P.W. Young and M. Giovannetti, 2012. Establishment, persistence and effectiveness of arbuscular mycorrhizal fungal inoculants in the field revealed using molecular genetic tracing and measurement of yield components. New Phytol., 194: 810-822.
51. Verbruggen, E., M.G.A. van der Heijden, J.T. Weedon, G.A. Kowalchuk and W.F.M. Roling, 2012. Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. Mol. Ecol., 21: 2341-2353.
52. Enkhtuya, B., J. Rydlova and M. Vosatka, 2000. Effectiveness of indigenous and non-indigenous isolates of arbuscular mycorrhizal fungi in soils from degraded ecosystems and man-made habitats. Applied Soil Ecol., 14: 201-211.
53. Smith, F.A., I. Jakobsen and S.E. Smith, 2000. Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. New Phytol., 147: 357-366.
54. Hart, M.M. and R.J. Reader, 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol., 153: 335-344.
55. Tchabi, A., D. Coyne, F. Hountondji, L. Lawouin, A. Wiemken and F. Oehl, 2010. Efficacy of indigenous arbuscular mycorrhizal fungi for promoting white yam (*Dioscorea rotundata*) growth in West Africa. Applied Soil Ecol., 45: 92-100.
56. Olawuyi, O.J., D.T. Ezekiel-Adewoyin, A.C. Odebode, D.A. Aina and G.E. Esenbamen, 2012. Effect of arbuscular mycorrhizal (*Glomus clarum*) and organomineral fertilizer on growth and yield performance of okra (*Abelmoschus esculentus*). Afr. J. Plant Sci., 6: 84-88.
57. Douds, D.D. Jr., P.E. Pfeffer and Y. Schachar-Hill, 2000. Carbon Partitioning, Cost and Metabolism of Arbuscular Mycorrhizas. In: Arbuscular Mycorrhizas: Physiology and Function, Kapulnik, Y. and D.D. Douds Jr. (Eds.). Kluwer Academic Publishers, Dordrecht, pp: 107-130.
58. Fortin, J.A., G. Becard, S. Declerck, Y. Dalpe, M. St-Arnaud, A.P. Coughlan and Y. Piche, 2002. Arbuscular mycorrhiza on root-organ cultures. Can. J. Bot., 80: 1-20.
59. Arora, N.K., D.K. Maheshwari and E. Khare, 2010. PGPR: Constraints in Bioformulation, Commercialization and Future Strategies. In: Bacteria and Plant Health, Maheshwari, D.K. (Ed.). Springer, The Netherland, pp: 97-116.
60. Wahab, S., 2009. Biotechnological approaches in the management of plant pests, diseases and weeds for Sustainable Agriculture. J. Biopesticides, 2: 115-134.
61. Cong, P.T., T.D. Dung, N.T. Hien, A.T.M.A. Choudhury and M.T. Rose *et al.*, 2011. Effects of a multistrain biofertilizer and phosphorus rates on nutrition and grain yield of paddy rice on a sandy soil in Southern Vietnam. J. Plant Nutr., 34: 1058-1069.
62. Stephens, J.H.G. and H.M. Rask, 2000. Inoculant production and formulation. Field Crops Res., 65: 249-258.
63. Keyser, H.H., P. Somasegaran and B.B. Bohlool, 1993. Rhizobial Ecology and Technology. In: Soil Microbial Ecology: Applications in Agricultural and Environmental Management, Metting, F.B. (Ed.). Marcel Dekker Inc., New York, ISBN-13: 9780824787370, pp: 205-226.

64. Smith, R.S., 1992. Legume inoculant formulation and application. *Can. J. Microbiol.*, 38: 485-492.
65. Redecker, D., H. Thierfelder and D. Werner, 1995. A new cultivation system for arbuscular mycorrhizal fungi on glass beads. *Angewandte Botanik*, 69: 189-191.
66. Plenchette, C., V. Furlan and J.A. Fortin, 1983. Responses of endomycorrhizal plants grown in a calcined montmorillonite clay to different levels of soluble phosphorus. I. Effect on growth and mycorrhizal development. *Can. J. Bot.*, 61: 1377-1383.
67. Millner, P.D. and D.G. Kitt, 1992. The Beltsville method for soilless production of vesicular-arbuscular mycorrhizal fungi. *Mycorrhiza*, 2: 9-15.
68. Malusa, E., L. Sas-Paszt and J. Ciesielska, 2012. Technologies for beneficial microorganisms inocula used as biofertilizers. *Scient. World J.* 10.1100/2012/491206
69. Declerck, S., D.G. Strullu and C. Plenchette, 1996. *In vitro* mass-production of the arbuscular mycorrhizal fungus, *Glomus versiforme*, associated with Ri T-DNA transformed carrot roots. *Mycol. Res.*, 100: 1237-1242.
70. Masso, C., J.R.A. Ochieng and B. Vanlauwe, 2015. Worldwide contrast in application of bio-fertilizers for sustainable agriculture: Lessons for Sub-Saharan Africa. *J. Biol. Agric. Healthcare*, 5: 34-50.
71. Brahma Prakash, G.P. and P.K. Sahu, 2012. Biofertilizers for sustainability. *J. Indian Inst. Sci.*, 92: 37-62.
72. Kumar, V., 2014. Characterization, bio-formulation development and shelf-life studies of locally isolated bio-fertilizer strains. *Octa J. Environ. Res.*, 2: 32-37.
73. Marin, M., 2006. Arbuscular Mycorrhizal Inoculation in Nursery Practice. In: *Handbook of Microbial Biofertilizers*, Rai, M. (Ed.). International Book Distributing Co., Lucknow, India, ISBN: 9781560222699, pp: 289-324.
74. Enti-Brown, S., P.O. Yeboah, S. Akoto-Bamford, A.K. Anim and H. Abole *et al.*, 2012. Quality control analysis of imported fertilizers used in Ghana: The macronutrients perspective. *Proc. Int. Acad. Ecol. Environ. Sci.*, 2: 27-40.
75. Jefwa, J.M., P. Pypers, M. Jemo, M. Thuita and E. Mutegi *et al.*, 2014. Do Commercial Biological and Chemical Products Increase Crop Yields and Economic Returns Under Smallholder Farmer Conditions? In: *Challenges and Opportunities for Agricultural Intensification of the Humid Highland Systems of Sub-Saharan Africa*, Vanlauwe, B., P. VanAsten and G. Blomme (Eds.). Springer, New York, USA, ISBN: 9783319076621, pp: 81-96.
76. Masso, C., J.M. Jefwa, M. Jemo, M. Thuita, D. Tarus and B. Vanlauwe, 2014. Impact of Inadequate Regulatory Frameworks on the Adoption of Bio-Fertilizer (e.g. PGPR) Technologies: A Case Study of Sub-Saharan Africa. In: *Recent Advances in Biofertilizers and Biofungicides (PGPR) for Sustainable Agriculture*, Reddy, M.S., R.I. Ilao, P.S. Faylon, W.D. Dar and R. Sayyed *et al.* (Eds.). Chapter 22, Cambridge Scholars Publishing, Cambridge, UK, ISBN-13: 9781443871051, pp: 258-268.
77. Tarbell, T.J. and R.E. Koske, 2007. Evaluation of commercial arbuscular mycorrhizal inocula in a sand/peat medium. *Mycorrhiza*, 18: 51-56.
78. Carrenho, R., S.F.B. Trufem, V.L.R. Bononi and E.S. Silva, 2007. The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. *Acta Botanica Brasiliica*, 21: 723-730.
79. Gaur, A. and A. Adholeya, 2002. Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. *Biol. Fertil. Soils*, 35: 214-218.
80. Gryndler, M., M. Vosatka, H. Hrselova, I. Chvatalova and J. Jansa, 2002. Interaction between arbuscular mycorrhizal fungi and cellulose in growth substrate. *Applied Soil Ecol.*, 19: 279-288.
81. Albertsen, A., S. Ravnskov, H. Green, D.F. Jensen and J. Larsen, 2006. Interactions between the external mycelium of the mycorrhizal fungus *Glomus intraradices* and other soil microorganisms as affected by organic matter. *Soil Biol. Biochem.*, 38: 1008-1014.
82. Van Der Heijden, M.G.A., 2010. Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. *Ecology*, 91: 1163-1171.
83. Mader, P., S. Edenhofer, T. Boller, A. Wiemken and U. Niggli, 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biol. Ferti. Soils*, 31: 150-156.
84. Kahiluoto, H., E. Ketoja, M. Vestberg and I. Saarela, 2001. Promotion of AM utilization through reduced P fertilization 2. field studies. *Plant Soil*, 231: 65-79.
85. Oehl, F., E. Sieverding, P. Mader, D. Dubois, K. Ineichen, T. Boller and A. Wiemken, 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. *Oecologia*, 138: 574-583.
86. Marschner, P., D.E. Crowley and C.H. Yang, 2004. Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. *Plant Soil*, 261: 199-208.
87. Johnson, N.C., 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytol.*, 185: 631-647.
88. Lopez-Raez, J.A., R. Matusova, C. Cardoso, M. Jamil and T. Charnikhova *et al.*, 2009. Strigolactones: Ecological significance and use as a target for parasitic plant control. *Pest Manage. Sci.*, 65: 471-477.
89. Garcia-Garrido, J.M., V. Lenzemo, V. Castellanos-Morales, S. Steinkellner and H. Vierheilig, 2009. Strigolactones, signals for parasitic plants and arbuscular mycorrhizal fungi. *Mycorrhiza*, 19: 449-459.
90. Mathimaran, N., R. Ruh, B. Jama, L. Verchot, E. Frossard and J. Jansa, 2007. Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsol. *Agric. Ecosyst. Environ.*, 119: 22-32.

91. Muchane, M.N., B. Jama, C. Othieno, R. Okalebo, D. Odee, J. Machua and J. Jansa, 2010. Influence of improved fallow systems and phosphorus application on arbuscular mycorrhizal fungi symbiosis in maize grown in Western Kenya. *Agrofor. Syst.*, 78: 139-150.
92. Kundu, C.A., 2012. Effect of arbuscular mycorrhizal fungi and phosphate solubilizing bacterial inoculants on growth and phosphorus uptake by orange fleshed sweetpotatoes (*Ipomoea batatas* (L.) Lam). Master's Thesis, The University of Nairobi, Kenya.
93. Kavoo-Mwangi, A.M., E.M. Kahangi, E. Ateka, J. Onguso, R.W. Mukhongo, E.K. Mwangi and J.M. Jefwa, 2013. Growth effects of microorganisms based commercial products inoculated to tissue cultured banana cultivated in three different soils in Kenya. *Applied Soil Ecol.*, 64: 152-162.
94. Mukhongo, R.W., M.A. Kavoo-Mwangi, M.E. Kahangi, E.M. Ateka and A.B. Were *et al.*, 2015. Occurrence of arbuscular mycorrhizal fungi and *Fusarium* in TC banana rhizosphere inoculated with microbiological products in different soils in Kenya. *Int. J. Soil Sci.*, 10: 45-62.
95. Wiseman, P.E., K.H. Colvin and C.E. Wells, 2009. Performance of mycorrhizal products marketed for woody landscape plants. *J. Environ. Hortic.*, 27: 41-50.
96. Antunes, P.M., A.M. Koch, K.E. Dunfield, M.M. Hart, A. Downing, M.C. Rillig and J.N. Klironomos, 2009. Influence of commercial inoculation with *Glomus intraradices* on the structure and functioning of an AM fungal community from an agricultural site. *Plant Soil*, 317: 257-266.
97. Corkidi, L., E.B. Allen, D. Merhaut, M.F. Allen, J. Downer, J. Bohn and M. Evans, 2004. Assessing the infectivity of commercial mycorrhizal inoculants in plant nursery conditions. *J. Environ. Hortic.*, 22: 149-154.
98. Monk, J., M. Tauschke, F. Eulenstein, A. Behrendt and C. Schneider *et al.*, 2014. Mycogro Ag[®] and Mycogro Hort[®] mycorrhiza products made in New Zealand. http://www.massey.ac.nz/~flrc/workshops/15/Manuscripts/Paper_Monk_2015.pdf
99. Sieverding, E., 1987. A VA-mycorrhizal fungus, *Glomus glomerulatum* sp. nov., with two hyphal attachments and spores formed only in sporocarps. *Mycotaxon*, 29: 73-79.
100. Sieverding, E., 1991. Vesicular-arbuscular mycorrhiza management in tropical agrosystems. *Deutsche Gesellschaft für Technische Zusammenarbeit, GTZ No. 224, Federal Republic of Germany*, pp: 371.
101. Bendavid-Val, R., H.D. Rabinowitch, J. Katan and Y. Kapulnik, 1997. Viability of VA-mycorrhizal fungi following soil solarization and fumigation. *Plant Soil*, 195: 185-193.
102. Schreiner, R.P., K.L. Ivors and J.N. Pinkerton, 2001. Soil solarization reduces arbuscular mycorrhizal fungi as a consequence of weed suppression. *Mycorrhiza*, 11: 273-277.
103. Gaur, A., 1997. Inoculum production technology development of vesicular-arbuscular mycorrhizae. Ph.D. Thesis, University of Delhi, New Dehli, India.
104. Gaur, A., A. Adholeya and K.G. Mukerji, 2000. On-farm production of VAM inoculum and vegetable crops in marginal soil amended with organic matter. *Trop. Agric.*, 77: 21-26.
105. Douds, Jr. D.D., G. Nagahashi, C. Reider and P.R. Hepperly, 2007. Inoculation with arbuscular mycorrhizal fungi increases the yield of potatoes in a high P soil. *Biol. Agric. Hortic.*, 25: 67-78.
106. Ingleby, K., 2007. Mycorrhizal training manual assessment of mycorrhizal diversity in soils and roots and nursery inoculation to improve the survival and growth of seedlings. Centre for Ecology and Hydrology, Bush Estate, Penicuik, Midlothian, UK., pp: 1-43.
107. Jarstfer, A.G. and D.M. Sylvia, 1994. Aeroponic Culture of VAM Fungi. In: *Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology*, Varma, A.K. and B. Hock (Eds.). Springer, New York, USA., pp: 427-441.
108. Raman, N., C. Sahadevan and V. Srinivasan, 2001. Growth of AM fungion *in vitro* root organ culture of *Sorghum vulgare* and *Saccharum officinarum*. *Indian J. Exp. Biol.*, 39: 1293-1298.
109. Declerck, S., D.G. Strullu and C. Plenchette, 1998. Monoxenic culture of the intraradical forms of *Glomus* sp., isolated from a tropical ecosystem: A proposed methodology for germplasm collection. *Mycologia*, 90: 579-585.
110. Chabot, S., G. Becard and Y. Piche, 1992. Life cycle of *Glomus intraradix* in root organ culture. *Mycologia*, 84: 315-321.
111. Declerck, S., D. D'Or, C. Bivort and F.A. de Souza, 2004. Development of extraradical mycelium of *Scutellospora reticulata* under root-organ culture: Spore production and function of auxiliary cells. *Mycol. Res.*, 108: 84-92.
112. Herrmann, L. and D. Lesueur, 2013. Challenges of formulation and quality of biofertilizers for successful inoculation. *Applied Microbiol. Biotechnol.*, 97: 8859-8873.
113. Smith, R.S., 1995. Inoculant Formulations and Applications to Meet Changing Needs. In: *Nitrogen Fixation: Fundamentals and Applications*, Tikhonovich, I.A., N.A. Provorov, V.I. Romanov and W.E. Newton (Eds.). Springer, The Netherlands, ISBN: 9789401103794, pp: 653-657.
114. Baylis, G.T.S., 1967. Experiments on the ecological significance of phycomycetous mycorrhizas. *New Phytol.*, 66: 231-243.
115. Mosse, B., C.L. Powell and D.S. Hayman, 1976. Plant growth responses to vesicular-arbuscular mycorrhiza. IX. Interactions between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. *New Phytol.*, 76: 331-342.
116. Menge, J.A., D. Steirle, D.J. Bagyaraj, E.L.V. Johnson and R.T. Leonard, 1978. Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. *New Phytol.*, 80: 575-578.

117. Jasper, D.A., A.D. Robson and L.K. Abbott, 1979. Phosphorus and the formation of vesicular-arbuscular mycorrhizas. *Soil Biol. Biochem.*, 11: 501-505.
118. Thompson, J.P., 1986. Soilless culture of vesicular-arbuscular mycorrhizae of cereals: Effects of nutrient concentration and nitrogen source. *Can. J. Bot.*, 64: 2282-2294.
119. Schubert, A. and D.S. Hayman, 1986. Plant growth responses to vesicular-arbuscular mycorrhiza: XVI. Effectiveness of different endophytes at different levels of soil phosphate. *New Phytol.*, 103: 79-90.
120. Amijee, F., B.P. Tinker and D.P. Stribley, 1989. The development of endomycorrhizal root systems: VII. A detailed study of effects of soil phosphorus on colonization. *New Phytol.*, 111: 435-446.
121. Sastry, M.S.R., A.K. Sharma and B.N. Johri, 2000. Effect of an AM fungal consortium and *Pseudomonas* on the growth and nutrient uptake of *Eucalyptus hybrid*. *Mycorrhiza*, 10: 55-61.