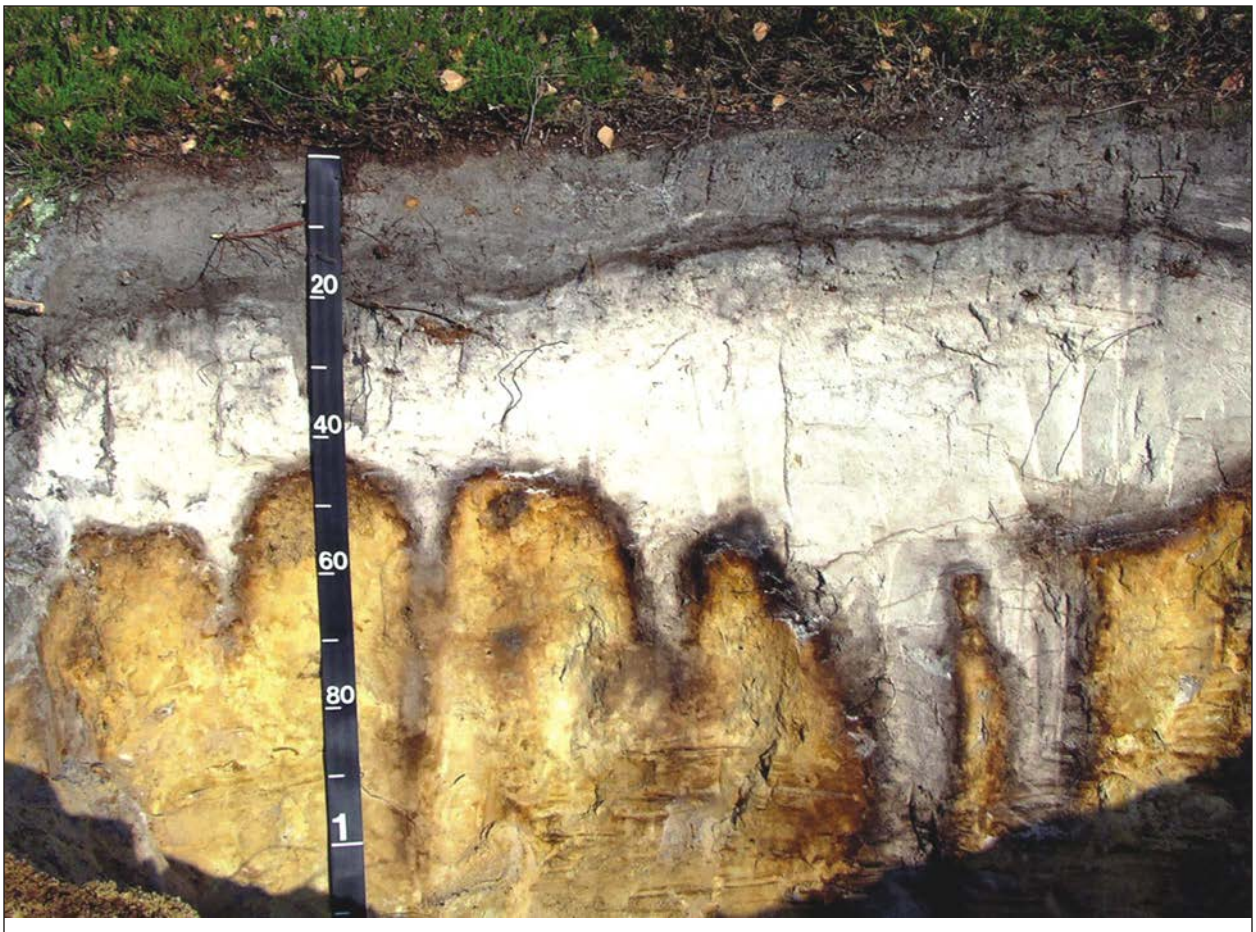

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Review Article
Production and Use of Arbuscular Mycorrhizal Fungi Inoculum in Sub-Saharan Africa: Challenges and Ways of Improving

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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

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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 crops[1]. 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 fertility[4].

Improved fallows were adopted in SSA for rapid replenishment of soil fertility[5] 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 irreversible[6] 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 inputs[7]. 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 countries[8]. However, in developing countries the great need for food due to population pressure implies a trend toward intensification and sustainable agriculture[9], 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 fertilizers[10]. Several studies have reported increased crop yield following application of rhizobial inoculants[11,12] and AMF[13-15]. Bio-inoculants are products containing living cells of different types of microorganisms with ability to mobilize nutrients for plant use through biological process[16].

Berg[17] 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 2020[18]. 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-inoculants[19]. 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* systems[21]. 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 owners[22] 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 harvested[23]. 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 conditions[24] (Table 2).

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

Bio-inoculant	Composition	Manufacturer	Reference
Rhizatech	*Glomus mosseae*	Dudutech, Naivasha, Kenya	Kundu[92]
Rhizatech	*Glomus etunicatum*	Dudutech, Naivasha, Kenya	
Rhizatech	*Glomus intraradices*	Dudutech, Naivasha, Kenya	
Rhizatech	*Glomus aggregatum*, *G. etunicatum*, *G. intraradices*, *G. mosseae*	Dudutech, Naivasha, Kenya	Kavoo-Mwangi *et al.*[93] and Mukhongo *et al.*[94]
Symbion vam plus	*Glomus* spp., *Gigaspora* spp., *Bacillus megaterium* var. *phosphaticum*	T. Stanes and Company Ltd., Peninsular, India	
Symbion vam	*Glomus fasciculatum*, other endomycorrhizal spp.	T. Stanes and Company Ltd., Peninsular, India	
Glomygel® Hortalizas	*Rhizophagus irregularis*	Mycovitro, Spain	
Mycormax	*Glomus* (2 spp.), ectomycorrhizal (ECM) (5 spp)	JH Biotec Inc., Ventera, US	Wiseman *et al.*[95]
BEI	*Glomus* (6 spp.), *Gigaspora* (1 sp.), *Paraglomus* (1 sp.)	Bio Organics Santa Maria, US	
AgBio endos	*Glomus* (6 spp.), *Gigaspora* spp.	AgBio Inc. WestMinister, US	
AM 120	*Glomus* (3 spp.)	Reforestation Technologies Int. Salinas, UK	
BioGrow endo	*Glomus* (3 spp.), *Trichoderma* spp.,	Mycorrhizal Applications Inc., Grants, US	
Die hard endo starter	*Glomus* (6 spp.), *Gigaspora* (9 spp.), *Trichoderma* (1 sp.)	Horticultural Alliance Inc., Sarasota, US	
Myco tree 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	
Myco super 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	*G. intraradices*	Premier Tech Biotechnologies, Canada	Antunes *et al.*[96]
Earth roots	AMF spp.	Not included	Corkidi *et al.*[97]
MycoApply endo	*G. intraradices*	Not included	
VAM 80	Not specified	Not included	
Ascend PB	Not specified	Not included	
NTC	Not specified	Not included	
Symbivit	*Glomus* 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 *et al.*[98]
Mycogro Hort®	Not specified	Fungi Perfecti®, Olympia, WA, New Zealand	
MycoApply endo	AMF (3 spp.)	Mycorrhizal Applications Inc., Grants, US	
MycoApply Micronized endo	AMF (3 spp.)	Mycorrhizal Applications Inc., Grants, US	
MycoApply root dip gel	AMF (12 spp.)	Mycorrhizal Applications Inc., Grants, US	
MycoApply endonet	*G. intraradices*	Mycorrhizal Applications Inc., Grants, US	
MycoApply soluble	7 AMF spp. (*G. intraradices*, *G. mosseae*, *G. aggregatum*, *G. clarum*, *G. deserticola*, *G. etunicatum*, *Gi. margarita*), 9 ECM spp. (*R. villosullus*, *R. luteolus*, *R. amylopogon*, *R. fulvigleba*, *P. tinctorius*, *Laccaria* (2 spp.), *Suillus* (2 spp.)), 2 *Trichoderma* spp. (*T. harzianum*, *T. konigii*), 19 bacterial spp. (*B. subtillus*, *B. licheniformis*, *B. azotoformans*, *B. megaterium*, *B. coagulans*, *B. pumlis*, *B. thuringiensis*, *B. stereothermiphilis*, *P. polymyxa*, *P. durum*, *P. florescence*, *P. gordonae*, *A. polymyxa*, *A. chroococcum*, *S. cervisiae*, *Steromyces griseues*, *S. lydicus*, *P. aureofaceans*, *D. erythromyxa*))	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 mycorhizal 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., *Bracharria decumbens* 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 *et al.*[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., *Sorghum sudanese*, *Zea mays* and *Daucus carota* may be frown 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 *et al.*[53] and Sieverding[99]
Nursery bed cultivation: Raised beds are prepared as in Gaur[103] and Douds *et al.*[57] 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. *Paspalum notatum* Flugge, 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 *in situ* 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 *et al.*[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 *et al.*[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	
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 producedi n 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 *et al.*[37] and Douds *et al.*[57]

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

	Nursery bed cultivation		Pot/Concrete tank cultivation		Aeroponic box cultivation		*In vitro* cultivation		
Factor	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	Gaur *et al.*[104]
Life cycle completion	Suitable for most	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	Douds *et al.*[105]
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	Ingleby[106]
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	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	Jarstfer and Sylvia[107]
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	Raman *et al.*[108]
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	Adholeya *et al.*[37]
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	Fortin *et al.*[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	IAEA[48]
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	Declerck *et al.*[109]

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 balances[25]. Secondly, tropical soils are highly weathered, acidic and characterized by P fixation. The AMF improve soil structure through particle binding[26] 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 phytopathogens[27-29]. At plant community level AMF reduce competition for water and nutrient thus influencing plant biodiversity[30] 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-specific[33]. Commonly observed benefits of AMF are improved uptake of nutrients especially P, but also ammonium (NH_4^+), 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 AMF[37], 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-inoculum[21] hence, poor development of the sector[38] 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 people[39], 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^{-1} [40]. 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 farmers[41-43]. Rhizobial bio-inoculants that are commonly used in SSA have demonstrated to economically increase yields in many part of the region[19]. 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 groundnut[44] 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, respectively[19]. 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 symbioses[45]. In Southern Africa, Chitedze Agricultural Research Station, Lilongwe in Malawi started producing commercial inoculants for soybean and cowpea in 1970s[46]. Zimbabwe has a large and well-established commercial BNF technology soybean sector[47] spearheaded by the Soil Productivity Research Laboratory (SPRL) and supported by the International Atomic Energy Agency[48]. 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 alternative[49] 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 harvested[23]. 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 m^2) using mycotrophic crops or in continuous pot cultures. Producing and maintaining monosporal 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 farmers[22] 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 situations[52]. Furthermore, a taxonomically functional diverse inoculum can be produced[53,54] as opposed to commercial inocula, which may contain only one species[49]. A formulation containing a consortium of AMF strains would have several advantages over single-isolate AM fungal inocula[37], 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 India[37]. *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 regions[59]. 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 technology[21], which has also negatively impacted development of the AMF production industry[38]. 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 commercialization[62]. 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 *et al.*[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 *et al.*[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 *et al.*[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 roots[49]. 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 capacity[63], 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 compounds[64]. 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 viability[23].

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 methods[23]. 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 level[65], expanded clay in the commercial sector[66], inert carriers such as sand, vermiculite, perlite and soil-rite (soilless compost)[67,68], powder, tablets/pellets or granules, gel beads and balls[37], alginate beads[69], 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 storage[59]. 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 farmers[70] 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 inoculants[59]. 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 considered[57]. 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 fragments[73]. 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 quality[74]. 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 Ethiopia[75], 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-inoculants[76]. 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 success[77]. 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 production[78], 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 colonisation[79]. 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 uptake[82], 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 diversity[83-85]. 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 branching[86] and allocate relatively more photosynthates to shoots and leaves instead of to the roots[87]. Reduced exudation results in low AMF colonisation and spore production[88-89]. 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 *et al.*[115]
Soils given 280 μg P g^{-1}	Decreased colonised root length and intensity of AM fungal biomass per colonised root length	Menge *et al.*[116] and Jasper *et al.*[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 *et al.*[120]
Soils with >25 mg P kg^{-1}	Greatest benefits of AMF	Sastry *et al.*[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 symbiosis[84], 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.

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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.

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