Chapter

Native Arbuscular Mycorrhizal Fungi and Agro-Industries in Arid Lands: Productions, Applications Strategies and Challenges

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Abstract

Bio-fertilizers based on mycorrhizal fungi represent a natural way to enrich the soil in respect of environmental balance. Arbuscular mycorrhizal fungi (AMF) are the most common symbiotic association between terrestrial plants and microorganisms, which are known to improve plants development and growth, especially under stress conditions. The potential for application of AMF in agricultures is an agro-ecological approach to allow better use of soil nutrient reserves. That receives increasing consideration for their prospective application for sustainable agriculture. The present chapter aims to highlight the agro-industrial strategy of AMF bio-fertilizers production explaining agronomics, ecological and economic approaches and benefits. This study aims to focus on the importance of production of bio-fertilizers based on indigenous AMF strains and their role in improving soils enrichment, which will subsequently lead to improved production and agricultural yields on degraded arid soils.

Keywords: degraded areas, native inocula, industrial production strategies, agro-economic benefits, conventional method

1. Introduction

Soils are considered as a dynamic system that contains varieties of microorganisms such as bacteria, actinomycetes, and fungi [1]. According to this richness in microorganisms, the eco-biological value of soil is considered. Whereas, maintaining this favorable soil microflora is very important for soil sustainability [2].

In the other hand, arid lands constitute about 35% of world land areas and are characterized by rainfall insufficiency, higher temperatures and evapotranspiration, lower humidity, and a general rareness of vegetation cover [3]. In return, a large mass of world's population lives in these areas, which it is imperative to nourish them. They practice livestock grazing and irrigated agriculture that they try to modernize in order to obtain the best yield. However, the agricultural techniques used in recent decades (use of large quantities of chemical inputs, soil compaction, etc.) which have caused in addition to soil degradation the decrease or even elimination of certain beneficial microorganisms from most cultivated soils, which has contributed to the loss of productivity of these soils [4]. This destitution requires regular additions in order to revitalize the soil and restore its productivity [2]. But what type of fertility for the soil? In agronomy, the notion of fertilization includes application of various chemicals products such as NPK chemical formulation, pesticides and herbicides, which further degrade the soil and reduce their duration [5]. In fact, modern agriculture are based on heavy usage of chemical fertilizers and harmful pesticides on the crops, with destruction of sustainability of the agricultural systems, cost of cultivation soared at a high rate, income of farmers stagnated and food security and safety became an intimidating challenge with considerable reduction in soil health [6]. In the best of cases where there is an interest in ecological stability, animal and plant waste is applied to fertilize the soils [5]. Recently bio-fertilizers notion begins to emerge. Bio-fertilizers are biological fertilizers based on symbiotic microorganisms. They are mainly divided into two groups: bio-fertilizers based on symbiotic bacteria and bio-fertilizers based on mycorrhizal fungi [7].

Bio-fertilizers are biological fertilizers based on plant symbiotic microorganisms, they are defined as a substance composed of living microorganisms which when applied to seed, plant surfaces, or soil colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the availability of primary nutrients to the host plant [6]. Usually, the bio-fertilizers are mainly divided into two groups: bio-fertilizers based on symbiotic bacteria and bio-fertilizers based on mycorrhizal fungi. Moreover, the presence in nature of bacteria solubilizing mineral elements is exploited; they are cultivated and used as a bio-fertilizer [4]. Furthermore, mycorrhizal fungi bio-fertilizers are divided on two types depending on the fungus itself; there are bio-fertilizers based on ectomycorrhizal fungi and bio-fertilizers based on arbuscular mycorrhizal fungi. Arbuscular mycorrhizal fungi (AMF) promote a significant increase of the area of root absorption of plants colonized, maximizing the use of water and nutrients [8, 9]. These symbiotic fungi enhance plant resistance to water stress, to high temperatures, improve resistance to conditions of toxicity and acidity of soil and to divers' type of pathogens [8]. In addition to soil stabilization in the form of aggregates [9]. AMF are known for their positive effects on phosphorus assimilation by the mineralization of organic phosphorus and solubilization of insoluble phosphorus [10]. In spite of their potential and benefits, the large-scale use of AMFs is still restricted, mainly due to the deficiency of availability of inoculant in high quantities, low cost and high quality, besides the lack of practicality of inoculation in the field [9, 11]. Their efficiency is also questioned by some authors [12, 13], who claim that AMF indigenous community promotes greater root colonization than the addition of commercial inoculants. In this context, the present study, aims to lift the veil on bio-fertilizers based on arbuscular mycorrhizal fungi to ameliorate agriculture in arid lands, their agro-ecological roles, technic of production and the challenges of possibility of installing a bio-fertilizer production unit in these areas.

2. Importance of arbuscular mycorrhizal fungi bio-fertilizers

Arbuscular mycorrhizas are the most common underground mutualistic symbiosis relationship [8]. They are considered as obligate biotrophic organisms that live in the metabolically active roots of terrestrial vascular plants, epiphytes, rhizoids and stems of bryophytes [1–8, 14]. Studies showed that AMF exists 460 million years before first plants originated [2, 15]. They form a mutualistic symbiosis between AMF, belonging to the Glomeromycotina sub-phylum, and 80%

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AMF species	Plant	Bio-fertilizers Nature	Stress type	Mechanism used	References
Glomus spp.	Tomato *Solanum hypersicum*	Commercial formulation	Drought	Improving water and nutrient absorption	Kuswandi and Sugiyarto [16]
Rhizophagus intraradices, *Funneliformis mosseae*	Orchard grass (*Dactylis glomerata*)			Improved water content	Kyriazopoulos et al. [17]
Glomus deserticola	*Ocimum basilicum*	Commercial formulation	Soil Salinity	Plant enhancement and alleviation of soil salinity	ElHindi et al. [18]
Mix of height AMF species originating from saline soils	*Tamarix articulata*	Indigenous formulation		Improving plant biomasses, water and nutrient absorption	Bencherif et al. [13]
Rhizophagus intraradices	Black locust (*Robinia pseudoacacia*)	Commercial formulation	Heavy metals pollution	Improved plant biomass causing positive impact on photosynthesis and macronutrient acquisition	Yong et al. [19]
Septoglomus constrictum, *Claroideoglomus lamellosum*, *F. geosporum*, and *F. mosseae*	Alfalfa (*Medicago sativa* L.) and tall fescue (*Festuca arundinacea* Schreb	Indigenous inoculum	Dioxin/furan polluted soils	Improvement of plant dry weight, bacterial, archaeal OUT 's and bacterial diversity	Meglouli et al. [20]
Rhizophagus irregularis, Funneliformis mosseae	*Triticum aestivum*. *Orvantis* and *Lord*	Commercial strain multiplied Commercial formulation	Biotic stress: Oïdium *Blumeria graminis*	Protection against pathogen and reduction of infection	Mustapha et al. [21]

Table 1.
Impact of AMF bio-fertilizers to alleviate biotic and abiotic stress.

of land plant species [8]. AMF are endophytic fungi with intra-radical hyphae that penetrate inside cortical cell and/or the root epidermis [7]. During this fungi and plant interaction, dialogues at molecular level take place, which result in host's metabolic modifications, protection against environmental stresses, and providing friendly conditions to symbiont (fungi) [2]. Arbuscules are formed by endomycorrhizal hyphae within the plant cortical cells and are highly branched, and mature arbuscules have short life and survive for 4–5 days. Arbuscules are considered as functional site for nutrient exchange [8]. These fungi benefit their hosts by increasing the uptake of nutrient elements (especially P) and enhancing the resistance to biotic or abiotic stresses [1, 7]. So far, a large number of publications demonstrated that AMF are an important regulator of the plants performance in different stressed environment (**Table 1**) such as heavy metal contained soils [20, 22], saline soils [13–15, 18, 20, 22, 23], soil subjected to drought stress [24]. AMF are also identified as regulator of biotic stress [21]. AMF association with plants not only improves plant growth but also improves soil texture by changing soil particles into stable aggregates that ultimately resists against wind and water erosion [15]. Mycorrhizal association is also helpful to introduce new plant species in new areas, occurring them ability to survive in a new environmental conditions [23]. Moreover, colonization of root by AMF can arise three sources of inoculum: spores, infected root and hyphae, collectively termed propagules [8].

As well as, with the emergence of sustainable development context the application of AMF bio-fertilizers starts to widen. It is assumed therefore, that the judicious use of these natural inoculants can reduce the need to amend soil with chemical fertilizers, thus increasing the viability of sustainable agriculture [15, 18]. **Figure 1** illustrates the role played by arbuscular mycorrhizal fungi in plant life cycle.

Thus selection of inoculum source constitutes an important parameter for the plantation successful. In fact, the response of AMF to abiotic stresses divers on the level of fungal species or ecotype; it was proven that indigenous strain occurs more beneficial effect into mitigates divers abiotic stress such as water deficiency, saline stress and heavy metals conditions by enhancing the active absorptive surface area which ultimately stimulates the uptake of water and nutrients [13–15, 20, 22, 23].

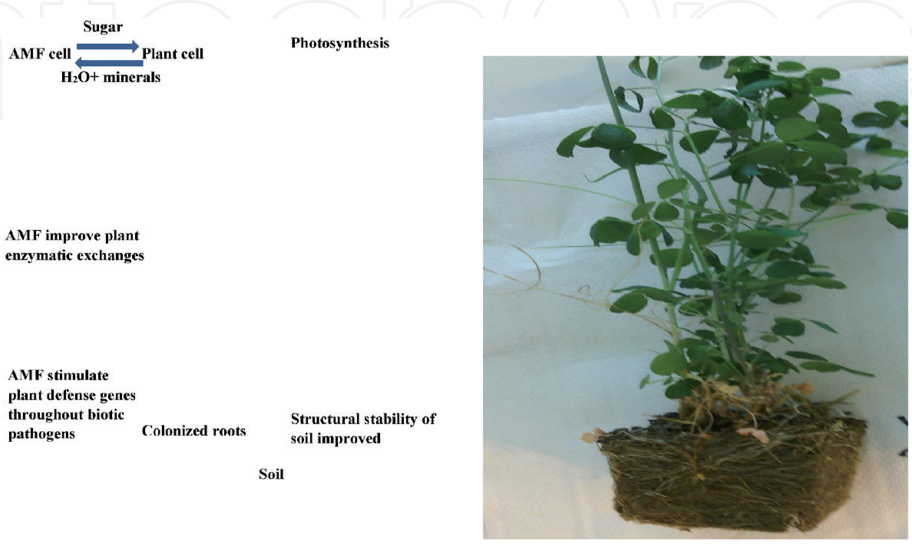

Figure 1.
Role and importance of AMF symbiosis in plant life.

3. Production of arbuscular mycorrhizal fungi bio-fertilizers

The commercial history of bio-fertilizers began with the launch of 'Nitragin' by Nobbe and Hiltner, a laboratory culture of Rhizobia in 1895, followed by the discovery of Azotobacter and then the blue green algae (BGA) and a host of other micro-organisms. Azospirillum and Arbuscular mycorrhizal fungi are fairly new discoveries [6]. Industrial manufacturing of AMF as crop inoculants is relatively new and, despite the practical demonstrations of their efficiency, their adoption by crop producers has been slow, most likely due to the quality and efficiency of marketed products [11]. In fact, production of previously selected AMF for their use as bio-fertilizers began in the decade of 1990 in a large part of the world [25]. Few companies throughout the world have manufactured and commercialized AMF inoculant using either a single AMF species or mixtures of AMF species that may include plant growth promoting rhizobacteria (PGPR) or other symbiotic and/or biocontrol fungi [11].

The production of inoculum differs from fungi family to another. Arbuscular mycorrhizal fungi are strict obligatory symbionts, they dependent on the presence of a host plant to accomplish their development and their multiplication. The inoculum producer is then required to co-cultivate the "fungus-host plant" complex. Without the use of host plants it would be impossible to complete the mycorrhizal life cycle until the production of new propagules/spores [7]. In addition to this monosporic inoculant, it is possible to produce inoculant with different native species with greater ease and speed [7]. In comparison with the commercial inoculant, it has a low cost, higher taxonomic diversity, and the use of locally adapted species [25, 26], which increases the chances of positive effects on the plant and avoid the introduction of exotic species [27]. The use of AMF inoculant produced from the forest soil is the most reliable and recommended method because of its high species diversity, the potential to accelerate the ecological restoration of the soil environment and to promote the germination and growth of the plants [9, 25–27].

3.1 Conventional method of AMF bio-fertilizers production

This method consists on AMF multiplication on pot culture with selective host plant under controlled conditions in a greenhouse or in a grow room [7].

3.1.1 Mixture of AMF species bio-fertilizers production

Native soil sampled from different plots of same natural sites must be mixed together to create one composite sample. The obtained mixture was distributed into pots (500 mL disposable cups) which were sown with trapping plant aiming to multiply and restore infective structures of the AMF species present in the trap cultures [27], and then kept in a greenhouse for four month. Two plant species are commonly used for trapping culture: cover "*Trifolum repens*" and leek "*Allium porrum*" [7, 27], But use of other legumes is also permitted such as Alfalfa "*Medicago sativa* [13] *Brachiaria* sp. [27]. Once the four month over, the areal part of plants is catted and the soils are mixed with roots for preparing a new plantation for other four months. Simultaneously, at each month, one pot of each plot was taken for analysis, using 50 g of soil for AMF spore isolation and identification and the roots for evaluation of the mycorrhizal colonization rate [26, 27]. A minimum of 12 months is required to obtain a good product, but the ideal is 24 months [13, 20]. Therefore, the obtained inoculums consist of different types of propagules: spores, fungal mycelium and fragments of mycorrhizal roots [7]. Multi-species products are closer to natural conditions because in ecosystems it is rare to encounter only one species of mycorrhizal fungus. The presence of several fungal species allows the inoculum to respond to a greater diversity of culture conditions.

3.1.2 Monospecific AMF species bio-fertilizers production

Production of monospecific AMF bio-fertilizer is based on the use of one selective AMF spore species isolated from natural soil using wet saving method [28], and cultivated on trapping culture with appropriate plant species on pot culture of 15 cm. Three months are needs to obtain AMF multiplication. Verification of AMF sporulation must be done each 20 days to one month. At same sanitary tests can also be performed to ensure that no contamination by parasitic fungi or sporulation of other AMF species has occurred. After four months, monospecific spores are ready for inoculation on seedlings of desired crops. In fact, [29] reported that Rhodes grass (*Chloris gayana*) is the best host for mass multiplication of *Glomus fasciculatum*. Bahia grass (*Paspalum notatum*) was used for multiplication of *Glomus deserticola* [27].

3.2 In vitro technic of AMF bio-fertilizers production

In vitro technic is an aseptic multiplication of AMF on roots cultivated on synthetic medium under sterile conditions. However, this technic started with the early work of [30], and subsequent development by [31, 32] and just after by [33], these authors developed the monoxenic cultivation system to produce contaminant-free AMF, allowing the realization of large-scale production under strictly controlled conditions [34]. The In vitro production of AMF bio-fertilizers consist on the extraction of potential viable propagules from soils with surface sterilization before optimization of growth conditions for germination under aseptic conditions. This aseptic technic consists on cultivation of number of AMF species in association with transformed host roots on synthetic growth medium [33]. Chabot et al. [35] established cultures from surface sterilized spores as starter material and produced 750 spores in 30 ml medium after a period of 4 months of growth in a mono-compartmental petri plate system. This is followed by the association of the propagules with a suitable excised host root for propagule production and recovery. Another system of in vitro AMF multiplication was developed by St Arnaud et al. [36], they used a bi-compartmental Petri plate system and obtained 15,000 spores in 3–4 months. Douds [37] improved this bi-compartmental system by replacing the medium in the distal compartment by fresh medium at regular intervals and obtained 65,000 spores in the distal side of the bi-compartment in a period of 7 months [34]. This technic of bi-compartment petri plate permitted to produce more than 250,000 propagules in 10 ml of medium, which made this technology attractive for industry. However, many process controls must be done to reduce the level of contamination, what should not exceed 3–5% [7, 34]. Once the AMF product is obtained; mass-produced propagules are then formulated in an utilizable form and stored before application to the target plant [7–34, 37]. This technic facilitates the efficient utilization of space and energy in the production system, using solid-state fermentation. Since the technology is more dependent on personnel, it lowers the number of man-days and achieves higher productivity [34].

The use of this technology remains useful for in vitro laboratory tests, but the mycorrhizal inoculum thus obtained (artificial environment on genetically modified roots) is not suited to applications in the agricultural field, providing overall unsatisfactory results [4].

3.3 Production of arbuscular mycorrhizal fungi bio-fertilizers in arid lands

Both conventional method and in vitro methods are practiced in arid areas to produce AMF bio-fertilizers. Several researches was focused on the increasing of plant production in arid land using AMF inoculum (**Table 2**), conventional method with AMF mixture was the most important technic of production adopted. Nevertheless, in vitro technic was also practiced such as by the energy and resource institute of India (TERI) [34]. They based on the faculty of Glomus genus to provide the possibility of using colonized roots as inoculum material with up to 80% of root colonization attained at 4 and 12 weeks [34, 39]. Despite, arid lands are often localized in underdeveloped country with low economical budget who cannot afford to allot enormous amounts in order to produce bio-fertilizers, so the conventional method remains the most appropriate technique under these conditions. In addition ecological conditions of arid lands give them specific characteristics that are not accommodating with all AMF strain. For that production of native AMF bio-fertilizers adapted to local conditions and to specific abiotic stress is essential [13]. Labidi et al. [39] developed a native AMF bio-fertilizer adapted to calcareous arid Tunisians soils. Abdelsalam et al. [38] produced AMF inoculum of desert saoudian areas using *Sorghum halepense* as trapping plant. Bencherif et al. [13] developed a specific AMF bio-fertilizer for arid saline soils. It is noted that in

Inoculum production technic	**Specific abiotic stress/zone**	**Propagule richness**	**Infection level**	**References**
Conventional method	Drought stress/Saoudian areas	20 g of Sudan grass rhizosphere with 950mycorrhizal spores and 0.5 g of colonized roots	78,5%	Abdelsalam et al. [38]
Conventional method	Calcareous/Tunisian areas	*Septoglomus constrictum*, *Funneliformis geosporum*, *Glomus fuegianum*, *Rhizophagus irregularis* et *Glomus* sp	90%	Labidi et al. [39]
In vitro method	Draught/Tafilalet Morocco	Rhizophagus irregularis	100%	Meddich et al. [5]
Conventional method	Draught/ Tafilalet- Morocco	Glomus sp., Sclerocystis sp., Acaulospora sp	15, 9, 1, spores, /gr of soil	Meddich et al. [5]
Conventional method	Heavy metal Polluted soil/Oran- Algeria	Acaulospora sp., Archaeospora sp., Glomus sp., Claroideoglomus sp., Ambispora sp., Diversispora sp	<50%, <20%, <5%,<5%, <5%/g of soil	Sidhoum and Fortas [40]
On-farm method	Drought	*Rhizophagus clarus* and *Claroideoglomus etunicatum*	80%	Moreira et al. [26]

Table 2.
AMF bio-fertilizers produced in arid lands.

all case, efficiency of AMF bio-fertilizers is related to the better combination AMF genotype/host plant genotype/adaptation to specific abiotic stress [13, 27].

3.3.1 On-farm method

To have AMF bio-fertilizer produced at a large scale with low cost, studies has been developed to test the multiplication of AMF spores under field conditions, called the "on-farm method". These studies explore AMF colonization with strains isolates that are environmentally adapted to native environmental conditions, which potentially representing a low-cost alternative for farmers [26, 37]. This technic is based on sowing plants seeds or seedlings in intact soil cores or mixed soil samples for sufficient time to allow development of AMF symbiosis and then roots were sampled, processed and assessed to measure mycorrhiza formation [26]. Indeed, [41] showed that multiplication on-farm with *Rhizophagus clarus* and *Claroideoglomus etunicatum* grown in agro-industrial residues, such as sugarcane bagasse, is a good strategy for the multiplication of AMF, leading to excellent inoculum potential and large numbers of spores. As well as, the on-farm technic allows farmers and nursery workers to access inoculums with the most effective AMF strains for their culture and their soil and climate conditions; furthermore, they can produce seedlings already inoculated with adapted AMF strains, which enhancing their establishment of s in the field conditions. In addition Moreira et al. [26] produced AMF inoculum with *Rhizophagus clarus*, *Claroideoglomus etunicatum* species, and native AMF from pineapple and coffee plantations, using spores multiplied by the on-farm method to enhance the growth of pineapple and coffee plantlets. These authors concluded that AMF inoculum favorite growth of the commercial tested crops with a high viability of AMF spores. This method is recommended for arid land due to their specific AMF strain and low coast, it could be applied and generalized in order to developing agricultural practices in these areas. Furthermore, because fungi carry different amounts of nutrients for plants, they may affect the growth of plants differently [37], it is preferable to use mixed AMF strains adapted to native conditions which could maximize the absorption of limiting nutrients [26]. This phenomenon could provide more benefits compared to colonization with exotic AMF strains or with single AMF species. Moreover, the mixed inoculation of AMF might have the characteristic of complementarity, exploiting the best of each species that colonizes the plant [26–37, 41].

3.4 Formulation of AMF bio-fertilizers

Formulation technologies largely take care of possible adverse environmental effects and factors that may render the inoculum ineffective [34]. In fact, bio-fertilizers are generally prepared as liquid suspension or more generally solid support containing different types of propagules: spores, fungal mycelium, mycorrhizal root fragments [7]. Generally AMF bio-fertilizers are presented with multi AMF species, which are closer to natural conditions; because in natural ecosystems it is rare to encounter only one species of mycorrhizal fungus. The presence of several fungal species allows the inoculum to respond to a greater diversity of culture conditions [13, 39]. Bio-fertilizer support can be composed of peat, vermiculite, lignite powder, clay, talc, rice bran, granulated rock phosphate, charcoal, soil, straw compost of rice or wheat otherwise a mixture of these materials. In current practice, for better framework life of the bio-fertilizer formulation, the support is selected on the basis of the viability of the microorganisms mixed with them. Likewise, the pre-sterilization of the support and its nutrient enrichment is the other strategy to improve framework, allowing AMF to be maintained in a non-competitive microenvironment. Sucrose, maltose, trehalose, molasses, glucose, and glycerol are additional nutrients and cell-protecting agents commonly used along with a support to ensure maximum cell viability and extended shelf life [4, 39]. After production, AMF bio-fertilizer may be in the form of granules, powder, tablets, pralins, or liquid suspension:

- The micro granules, between 1 and 4 mm, are easily mixed with support of mycorrhizal plants or brought into the planting surface as close as possible to the roots;

- Very fine powders (particles <250 μm) make it possible to prepare a suspension which can be sprayed on growing media or injected into the soil at the base of plants already installed. This type of inoculum can also be used as a seed coating.

- The tablets allow easy dosing of the inoculum to be placed in the plantation area. The inoculum provided is localized in one place and not distributed evenly over the roots.

- Associated with a praline, the inoculum is particularly suitable for plants with bare roots. In a single operation, the plant is inoculated and its roots protected.

- Liquid suspensions are suitable for coating seeds. These inocula can also be sprayed on growing media or injected into the soil at the base of plants [4–7, 39].

The formulated AMF bio-fertilizers should be positioned near the roots, with avoiding the spreading products and favor the injection or burying method. Finally, it is also possible to produce "2 in 1" products:

- Inoculated planting support, ready to use and particularly suitable for soil-less culture,

    - Pre-inoculated plants (vine, chestnut, etc.) ready for planting and whose mycorrhization has been checked before marketing,

    - Seeds coated with AMF propagules (mainly spores) which make it possible to spread and inoculate a plot in a single pass,

    - Organic fertilizers and amendments containing AMF propagules [7].

4. Design of AMF bio-fertilizer production unit

The aim of our present study is to set up a production unit for AMF bio-fertilizers, which has the advantage of covering the biological deficit of arid soils, a problem which continues to degenerate more and more. Indeed, the installation of a bio-fertilizer unit must follow some criteria such as: appropriate production, location, construction space, equipment, machinery, other laboratory equipment and working capital. The bio-fertilizer production unit should be founded in a homogeneous area based on the interaction of soil characteristics, geomorphology and climate. This place should be qualified as a buffer to reduce the risk of contamination during the process of production and quality control. The overall architecture of the unit is the key element for the success of any economic project. In our case, the bio-fertilizer production unit must be organized in an H-shaped architecture (**Figure 2**). This comes, according to Alamari [4], This author explained that this structure is based on the fact of its economical aspect and its ability to adapt to the sterilization process with forward walking, in other words from the "soiled", to go towards the "clean" and then towards the "sterile", without possibility of going back and without crossing of flows of "soiled" and "clean". The unit must contain administration, laboratories, storage and packing space.

4.1 Administration

The administrative team must ensure the respect of directives and guidelines in addition to external relations allowing the best conditions for the scientific team to carry out their work. To achieve these objectives, different tasks must be implemented: (i) Preparation, application and management of the budget and control of its execution; (ii) Establishment of contracts and agreements with different organizations in the same field, while taking care of calls for submissions and the

Figure 2.
Conventional method for production of mixed AMF bio-fertilizers

various procedures for procuring equipment; (iii) Physical and telephonic reception is provided; in addition to management of the staff and personnel of the unity by application and monitoring of health and safety guidelines.

4.2 Laboratories

The setting up of a laboratory should meet the criteria approved by the World Health Organization (WHO). A laboratory should be built with walls, ceilings and horizontal surfaces, non-polished, easy to clean, impermeable to liquids and resistant to disinfectants and to antiseptics. Then, to ensure the best work conditions, the laboratory areas must be spacious [42]. In addition, the laboratory must contain:

- Mechanical ventilation system ensuring interior air movement without recycling;
- Electricity must be sufficiently powerful with an emergency restart system in the event of blackouts;
- The town gas supply must be appropriate and protected.
- Presence of the cleaning tank with emergency shower next to each laboratory door, with installation of physical protection and fire safety systems.
- Finally, providing enough materiel resources and space for treatment and safe storage of solvents, radioactive substances as well as compressed and liquefied gases.

The production unit laboratory of AMF bio-fertilizer must contain three compartments: (i) Greenhouse for AMF inoculum multiplication; (ii) In vitro multiplication and strains isolation rooms; and (iii) compartment of control, conditioning and storage.

4.2.1 Greenhouse AMF multiplication

The greenhouse for AMF multiplication must be positioned behind the unit occupying a clear space with 204 m^2 of approximately area. The greenhouse is used to care for young host plants and to maximize crop productivity by improving the relationship between their growth and AMF biomass. Greenhouse is the most important compartment of the unit, so the geographical location may have to be considered. The attractive location must be related to the adaptability and value of the land, cost of fuel delivered, ample and inexpensive water, in response of number of question: (i) What is the yearly available solar energy? (ii) How much moisture falls, summer and winter? (iii) What are the maximum and minimum temperatures and their duration? (vi) What are the hail and wind belts?(v) Is air pollution a potential problem? (vi) Information on all of the foregoing questions allows the greenhouse operator to determine the degree to which he can maintain near optimum environmental conditions for plant growth and AMF multiplication [43]. Wind is important climatic problem in arid lands, so the wind direction plays an important role in the choice of unit implantation site. So, orientation of the greenhouse is a compromise for wind direction, latitude of location and type of temperature control [44]. After site choice, the greenhouse must follow some recommendations. The greenhouse must be constructed in glass farmed structure on double-sloped with a naturally exposition of natural light for much of the day.

In addition, heat is partly assured by sun rays paired with artificial means, such as circulating steam, hot water, or hot air. Ventilating system is also needed [43, 44]. For low coast, the ventilation must be assured by roof openings and large windows on the side, which can functioned mechanically or automatically. In some conditions, if a financial condition allows automatic ventilation system is installed. For the AMF multiplication, trapping culture must be done in pots or in specialized containers. In this setting, trapping cultures are grown for 3 to 4 months to minimize the accumulation of saprophytes in the medium for excessive growth and senescence of the roots. However, culture maintained for more than 5 months and regulated watering is recommended before areal part cutting and replantation of new seeds as explained above (3.1.1).

4.2.2 In vitro multiplication room

It represent an aseptic areas separated from the greenhouse and the AMF isolation room in order to avoid any contamination and to control sterility conditions as much as possible.

4.2.3 Drying and conditioning room

This area is located just before the greenhouse; it is used to dry the contents of the pots and containers for later conditioning. Once the trapping plants are ready to be harvested, they are moved to shelves in this area so that they are not exposed to light. Drying take about 2 to 3 weeks. After this period, the roots of the trapping plants are cut and mixed with a suitable substrate. Conditioning AMF inocula begins by placing the cultures in sealed bags. These bags are provided with codes written both on the surface and on labels affixed to the upper left corner of the crops so that they are easily identified when stacking. In addition, an organization in alphabetical order of cultures is also recommended.

4.2.4 AMF strain isolation room

Isolation room is completely isolated from all plant growing areas and the use of unsterilized soil is strictly prohibited. Isolation is practiced as follows: The contents of the dried pots are installed on grounds. The isolation of the spores from the sample is done by wet sieving method [28]. This technic of isolation is practiced in order to produce bio-fertilizer containing AMF spores. In addition isolated AMF strains are conserved in order to develop further research. During the AMF isolation process, a series of precautions must be observed, especially disinfection of surface area of isolation, tables and shelves with draying after cleaning. Asepsis is main condition for the success of this crucial stage of the AMF bio-fertilizer production.

4.2.5 Control room

This space is used for carrying a series of bio-fertilizer control tests. These tests include AMF spores count with microscopic examination, evaluation of AMF root colonization rate and elaboration of must probable number test. AMF spores number must vary between 10 and 15 pots per day. Once extracted, the spores are transported in glass Petri dishes and stored in the laboratory refrigerator. Indeed, the examination is carried out by a stereo microscope on the day of the extraction. The information thus retrieved is stored in a database and all written notes are archived as a physical backup. Systematically, all the files having processed the culture collection are stored centrally on the unit's web server, saved on a separate hard drive on the same computer, and stored on another computer in the laboratory.

4.2.6 Storage room

The storage is done at an atmosphere of 4°C at the level of the shelves of metal racks. These are characterized by mesh surfaces to optimize air circulation and facilitating their cleaning. The racks are placed in the center of the room and equipped with wheels to facilitate their movement. The storage period can reach a maximum of 3 years [4]. Bio-fertilizer storage process requires certain recommendations mainly: bag surface and their labels must be cleaned and disinfected before they are placed in this room. Floors and shelves are regularly disinfected. AMF bio-fertilizer product should be stored in a corner of the laboratory where the air temperature is not detrimental to the viability of AMF propagules.

5. AMF bio-fertilizers challenges of production and application on agricultural projects of arid lands

5.1 Why produce native AMF bio-fertilizers for arid lands?

Arid lands constitute the most widespread terrestrial biome in earth, with 35% of the land areas of the world. These areas are subjected to several desertification phenomena [3]. To counteract this problem, applications of new agricultural technics are required including application of bio-fertilizers. Nevertheless, the use of AMF for the restoration of degraded ecosystems has received poor attention, requiring a different approach [25]. In addition, loss of AMF propagules is usually recorded following soil and cover plant degradation, which could further inhibit natural and/or artificial revegetation processes [3, 25]. Taking into account all the previously cited aspects and the necessity of restoration in these areas, the eco-technology proposed by some studies [13–15, 18, 20, 22, 23, 25] represents a good alternative. They propose the restoration of degraded areas by re-introduction of native AMF and plant species [25].

5.2 How produce native AMF bio-fertilizer unit?

Production of native AMF bio-fertilizers unit require appropriate funding with adoption of a good financing strategy, based on various technical-economic parameters including description of the income elements and those of the expenses (**Table 3**). Indeed, the elements of income include the sale of bio-fertilizers, remunerations, publicity and assurance. **Table 4** describes all the expenses and revenues provided by the bio-fertilizer production unit.

5.3 Economical challenge of native AMF bio-fertilizer production?

Production of native AMF production is an agro-industrial investment for each arid region, in some case the product may be exported to similar edapho-climatic areas. Designed production and marketing chain must support each unit in order to guarantee the success of the project. In **Table 4** we have tried to establish an approximate economic study which will allow the investors to have an idea on the economic situation of the project, but these figures may vary from one country

Space	Surface (m^2)	Realization coast ($)
Reception	35.61	91,873,800
Conferences room	31.91	82,327,800
Manager's office	17.3	44,634,000
Engineers office	56.02	144,531,600
Storage room	55.13	13,235,400
Isolation room	40.55	104,619,000
Multiplication room	61.88	524,436,600
Conditioning room	64.6	1,666,668,000
Quality Control room	97.99	252,814,200
AMF greenhouse multiplication	203.27	655,545,750
Sales office	41.63	107,405,400
Store checkout	44.34	114,397,200
Changing room and toilets	73.23	188,933,400
Total	823,46	3,991,422,150

Table 3.
Realization coast of native AMF bio-fertilizers unit.

Expenses ($)			Incomes
Investment costs	Construction field	65,000/m^2 in average	24$/kg of Native AMF bio-fertilizer
	Construction	**4,000,000,000**	
	Technical installation	40,000	
	Materials	100,000	
	Tools	100,000	
	Transport	100,000	
Production charge	Raw material production	13,500,000	
	Packaging	9,030,000	
	Salaries	Varied between 3,225,000 and 10,320,000/employee	
	Repair and maintenance	10%	
	Insurance	10% of the operating budget	
	Publicity	10%	
Supplementary charge		2–3%	
Total	850 milliards of dollars of investment	12900 millions of dollars /year	

Table 4.
Expenses and revenues of native AMF bio-fertilizers unit.

to another. The main thing is to ensuring the success of the plant development, potentiates the in situ conservation of the AMF community and preservation of ecosystem stability and biodiversity (**Figure 3**).

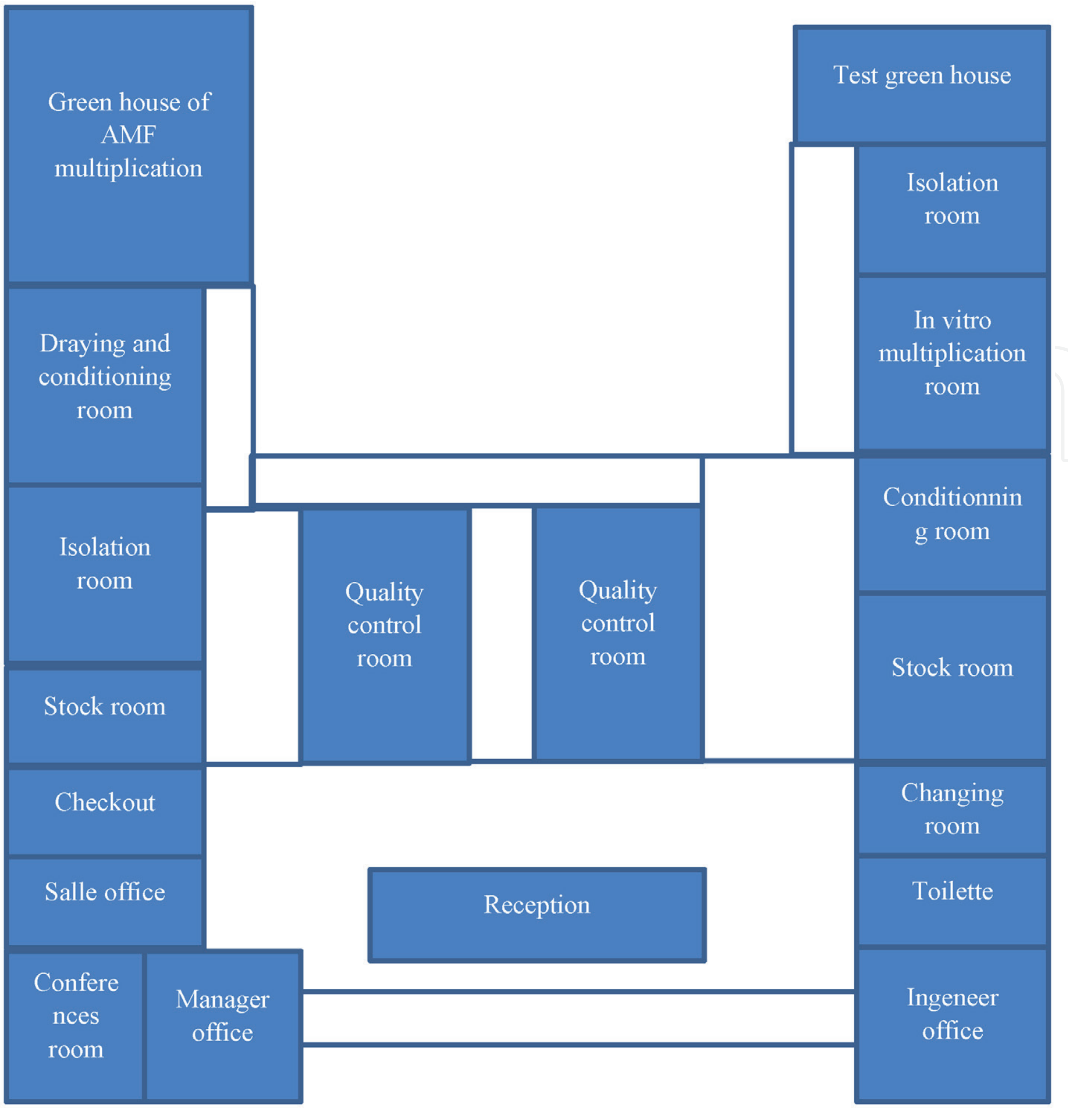

Figure 3.
Representative plan of AMF production unit on H form (Adapted from Alamri [4] description).

In absence of information about the stapes and strategies of native AMF bio-fertilizer unit, these information given in **Tables 3** and **4** can help in understanding and progressing of this strategy and its application in degraded arid lands for development of sustainable agriculture.

6. Conclusion

Arid land has specific ecological characteristics that confer them special management strategies. As same the different soil throughout the world, soil of arid land present a diversity of AMF that may be exploited to enhance agriculture in these areas. For that in the present chapter we have exposed the most important technic of AMF bio-fertilizer production based on native AMF strains. Founded on what has been explained, recommending the use of conventional pot culture technic present the adequate method adapted for arid lands. We have also given an approximate economical evaluation for coast of building and installing native AMF production unit in arid lands. The conventional method includes optimization of scale of production while using the adequate trap plant, law-coast and environmentally safe. With this technology, the preservation of environment from chemical fertilizer pollution must be enhanced, which lead to economic development of agro-industry adapted for each country with operative system for sustainable agriculture.

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Conflict of interest

The authors declare no conflict of interest.

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