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A bioeconomic Approach for the Production of Biofertilizers and their influence on Faba Bean (*Vicia faba* L) Productivity

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Abstract

Nowadays humanity faces severe environmental problems, due to the large amounts produced of organic waste from industry, agriculture and household, besides the sewage sludge from wastewater treatment plants; and, the loss of soil fertility due, in part, to the extended use of chemical fertilizers. However, bioeconomy takes advantage of biological resources or bioprocesses for the production of value added products; in this sense, we evaluate two fertilizers, an inoculum of Arbuscular Mycorrhizal Fungi (AMF), and a compost prepared with sludge from two wastewater treatment plants, agriculture residues and chile waste. They were tested sole and as a mixture, over the growth of faba bean. Application of AMF with or without compost, increased the plant height, fresh and dry biomass of foliage and fresh root biomass, compared to the use of compost or soil. In the presence of AMF the fresh biomass was increased by 39% and with compost 26% compared with control. The application of biofertilizers, so it is necessary to promote their use.

Keywords: Arbuscular mycorrhizal Fungi, compost, faba bean, biofertilizer

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1. Introduction

Nowadays humanity faces severe environmental problems due to the large amounts of organic waste produced by cities, industries and farms, which are hard to be discharged, besides the serious threat that represents to the environment, human health, and toxicity to beneficial microflora in soil (Ahmad et al., 2007).

Annually, enormous amounts of food supply chain waste are generated and these wastes have impact on climate, water, land and biodiversity, besides constitute an economic and social problem (FAO, 2013). Food waste is generated in every step along the life cycle of food supply chains, starting from agricultural production and post harvest practices to industrial processing, whole sale/retail sector, food service sector, and household consumption (FAO, 2013; Turon et al., 2014).

As the population and industry grow, water pollution becomes an increasing serious problem around the world that has made imperative the implementation of wastewater treatments. Sludge is originated during the process of waste water treatment; it concentrates heavy metals, poorly biodegradable trace organic compounds and potentially pathogenic organisms, such as helminth eggs, fecal coliforms, salmonella, protozoan cysts and viruses. There is an increase in the amount of sewage sludge produced annually, for example in the European Community the annual production in 1992, was of 5.5 million tons of dry matter, and by 2005 was nearly 9 million tons. Then the generation of sludge is a rising concern, because due to the large quantities produced and its dangerous properties must be treated in such a way that generates a safe product. However, sludge is rich in nutrients such as nitrogen and phosphorous and contains valuable organic matter, that can be useful when applied to soils that are depleted or subject to erosion (European Commission, 2015).

Agriculture has become an alternative destination for the substantial quantities of sewage sludge and food waste, due to the presence of plant nutrients in these materials. Nevertheless, direct application of sewage sludge can harm the soil-plant system, due principally to an imbalance in nutrients and because this waste can provide significant amounts of potentially toxic elements and pathogens. Such contaminants can be more available to plants and be accumulated in the soil (Moretti et al., 2015). For the sustainable agricultural use of sewage sludge and organic waste, it is necessary that their properties are safe enough to be used without harm the environment. To accomplish these requirements, they must be subjected to an adequate post-treatment, to improve its chemical, physical, and microbiological properties, like composting (Moretti et al., 2015).

The composting process is characterized by aerobic decomposition of materials like sewage sludge, food and agriculture waste. The use of compost reduces the risks of environmental contamination as compared to the direct use of sewage sludge or food waste, because composting helps to reduce the levels of heavy metals, diminish the risk of nitrate leaching, and promote the eradication of pathogens. The humic substances formed during the composting process stimulate the building of soil fertility, and there is an actual increase in organic matter content in the soil (Ahmad et al., 2007; Moretti et al., 2015; Viaene et al., 2015; Hargreaves et al., 2008; Vakili et al., 2015).

1.1 Bioeconomy

Bioeconomy is the economic activity that takes advantage of biological resources or bioprocesses for the production of value added products such as food, fuels, chemicals, bio-based products and bioenergy. Bio-based systems can be exploited sustainably for economic gain, a waste is something that costs you money to have taken away, but underutilized wastes have the potential to be converted into high value products; in this way, waste is not only an economic opportunity, but offers a potential low carbon alternative to petrochemicals and materials obtained directly from nature in their raw form (OECD, 2009).

The transformation of waste can make an important contribution to stimulating a bioeconomy, and can therefore be transformed from a problem into a resource. A waste based bioeconomy could deliver substantial economic returns and support a considerable number of jobs (House of Lords, 2014). Bioeconomy relies on renewable biomass instead of finite fossil inputs for the production of value-added products such as food, biobased products and bioenergy (Viaene et al., 2015). Renewable biomass can be obtained from food crops, grasses, trees, household, industrial and agricultural waste and sewage sludge, that can be used to achieve sustainable production of products like paper, biofuels, plastics, biofertilizers and industrial chemicals, with the aid of biotechnology (House of Lords, 2014; OECD, 2009).

1.3 Compost

There has been a decrease in agriculture productivity, due to nutrient deficiency of soil, build of obnoxious weeds and pests, and increasing cost of production over the last two decades, then the growth rate in agriculture is not keeping pace with the population growth rate. The increasing population pressure has forced many countries to use chemicals fertilizers to increase the farm productivity at a required level (Babu et al., 2014).

Intensive agricultural practices such as the use of chemical fertilizers, frequent soil tillage, narrow crop rotations improve the production in short time; however, in the long run resulted in decreases in the organic carbon in soil, soil aggregation strength, biodiversity loss, soil erosion, which diminish the productivity of field crops, besides pollution of ground water and air (Vakili et al., 2015; Viaene et al., 2015; Yadav et al., 2013).

The quality of soil is associated with the degree of fitness of a soil for a specific purpose, also, is understood as a series of ecological functions, such as nutrient cycling, energy and water storage, and biological transformations (Calleja-Cervantes et al., 2015). One of the most important characteristics of soil fertility is sufficient soil organic carbon (Viaene et al., 2015). Land application of organic wastes has emerged as an attractive and cost effective strategy; these wastes have been proved to supply plant nutrients and organic matter to the soil for crop production. However the direct application of raw organic wastes is inappropriate for land and agricultural production (Ahmad et al., 2007). Solid urban waste, food factory waste and other industrial by-products, sewage sludge, agricultural residues and domestic waste contain sufficient amount of organic materials for composting. Composting is considered the most appropriate option for addressing the constraints associated with organic solid waste materials for agricultural use, it is considered as one of the most favorable, cheap, and simple methods used to treat and stabilize these kinds of waste (Vakili et al., 2015).

Unlike fast-release fertilizers, such as mineral fertilizers and slurry, compost contains large amounts of organic matter, which enhances the soil organic carbon content; compost is also a source of nutrients, which reduces the need for other fertilizers, then diminishes both the cost of purchasing nonorganic fertilizers and the environmental impact associated with fertilizer production and use (Vakili et al., 2015).

Compost can play an important role in the bioeconomy because its production does not rely on finite inputs. It is an economically attractive technique of waste disposal and recovery of valuable plant nutrients, improve soil biophysical properties, soil organic matter and crop yields (Ahmad et al., 2007); besides compost can be produced locally on the farm (Viaene et al., 2015). In composting there is a reduction in the volume of the waste, kills pathogens that may be present, decreases germination of weeds in agricultural fields, destroys malodorous compounds and stabilizes the nutrients, mature organic matter and produce usable environmentally friendly final product (Ahmad et al., 2007; Viaene et al., 2015; Hargreaves et al., 2008; Vakili et al., 2015).

Compost application has well-established beneficial impacts on soil quality, soil fertility and the environment (Viaene et al., 2015), is also gaining popularity because of its positive effect on biological, physical, and chemical soil properties (Hargreaves et al., 2008), like available water content and aggregate stability, which in turn protects the soil against erosion (House of Lords, 2014).

The nutrients in compost are released gradually because they are already fixed in the microbial biomass; compost application therefore helps to prevent nutrient leaching to groundwater and contributes to soil fertility in the long term. Additionally, repeated compost amendments can enhance the beneficial soil microorganisms of the soil, total carbon, and cation exchange capacity, can decrease the amount and relative abundance of plant-parasitic nematodes and lowered down bulk densities, thus improved soil quality. The risk of plant diseases might be reduced and thus the use of pesticides and herbicides (Yadav et al., 2013; House of Lords, 2014).

According to Ahmad et al. (2007), all materials that can be naturally changed by microorganisms into environmentally friendly substances are compostable. But there are many factors that affect the composting process, such as the aeration rate, oxygen consumption rates, compost recycling, moisture content and pH (Luet al., 2009).One of the most important aspects of the total nutrient balance is the ratio of organic carbon to total nitrogen (C/N), a high C/N ratio is known to diminish biological activity and slow the composting process; whereas a low C/N leads to loss of nitrogen as ammonium. An initial C/N ratio of 25/1 to 30/1 is considered as optimum for composting. Water is essential for all microbial activity and should be present in appropriate amounts throughout the composting cycle.

Sewage sludge can hardly be composted alone, because of its compacted structure, high water content, and low C/N ratio, then sewage sludge needs to be mixed with dry and carbon-rich materials like food industry waste, sawdust, vegetal remains, straw and wood chips, which act as bulking agents, absorbing the moisture and providing the composting mass with an appropriate degree of sponginess and aeration; the end product enables adding a significant amount of carbon without adding large amounts of phosphorus and nitrogen (Ammari et al., 2012; Zhao et al., 2015; Banegas et al., 2007; Yousefiet al., 2013). Compost with a C/N ratio between 9/1 and 10/1 is considered as mature, also is recommended a C/N ratio below 16/1, to avoid nitrogen blockade due to competition between microorganisms and plants (Ammari et al., 2012).

The use of compost and some microorganisms present in soil, like arbuscular mycorrhizal fungi, have demonstrated to enhance the soil productivity in a sustainable manner. Then it is important to consider the application of both fertilizers to improve the agricultural productivity in a sustainable way.

1.4 Arbuscular mycorrhizal fungi (AMF)

Microbiota plays an important role in agriculture since it contributes to soil fertility, improves soil structure and biodiversity and has a real effect on plant development. Among these microorganisms are the symbiotic arbuscular mycorrhizal fungi, belonging to the phylum *Glomeromycota*. Mycorrhizal is the symbiotic association between plant roots and the hyphae of soil-specialized fungi, and it is considered the principal organ involved in nutrient uptake by most terrestrial plants, increasing the capture of phosphorus, calcium, copper, sulfur, zinc and iron. This association plays a very important role in the effective exploitation of mineral resources in the soil and in the protection of roots against a number of pathogens. Mycorrhizae are therefore essential for the survival of many plants in diverse ecosystems, including many crops (Larcher, 2003; Yao et al., 2002).

AMF have been reported to protect plant from some root infecting fungi and are therefore potential biological control agents; biocontrol methods can be an option for disease control and growth enhancement. Interest in the ability of beneficial microorganisms to control diseases has grown, particularly with respect to that environmentally friendly. The inoculation of some crops with AMF has helped to control disease; for example, inoculation of tomato seedlings with *T. harzianum* and AMF significantly enhances the growth of tomatoes and control the disease caused by Fusarium, *M. javanica* and nematode *Nacobbusaberrans;* a similar situation was observed with *G. etunicatum* in kiwi (*Actinidiadelicios* Chev.), attacked by *M. javanica*. (Mwangi et al., 2011; Marro et al., 2014; Banerjee et al., 2013). The AMF, applied on *G. intraradices* increased plant height, leaf and root dry biomass, panicle length, and protein content in the grain of sorghum (Diaz-Franco et al., 2008).

Lately, much effort has been paid to get suitable formulations for AMF inoculum and appropriate ways for their application to the soil.

The study and selection of isolates from endemic AMF communities could represent an alternative to the use of not native organisms, also in the aim of restocking those soils with a microflora depleted by intensive agricultural practices (Sasvári et al., 2012; Camarena-Gutiérrez, 2012).

1.5 Faba bean (*Vicia faba* L)

Faba bean is a significant source of protein rich food and the nutritional value is high, in some areas is considered to be superior to peas or other grain legumes. Faba bean is used both as a human food and a feed for pigs, horses, poultry and pigeons (Jensen et al., 2010). Besides, the various parts of the plant have been widely used as feed in order to improve animal performance. Faba bean cultivars used for dry grain production leave a considerable amount of harvest residues, mostly in the form of thick dry stems, that are suitable for green manure; because their low lignin and other harder degradable compounds positively affects the release of nitrogen from the biomass in the soil, thus increase its fertility and have long-term positive impacts upon succeeding crops. In recent years faba bean has been widely used in ecological agriculture (CemToprЭкспо 2011-2015; Multari et al., 2015).

The objective of this work was the preparation of three biofertilizers: compost from sewage sludge, food and agriculture waste; inoculum of AMF isolated from soil; and, a mixture of the compost and AMF. The three biofertilizers were used to fertilize soil to determine their efficiency over the growth of faba bean.

2. Materials and Methods

2.1. Isolation of the arbuscular mycorrhizal fungi (AMF), quantification and Morphological analyses.

100 g of soil were suspended in 900 mL of water and thoroughly mixed, then settled for 4-5 min, AMF spores were isolated from the mixture through sieves (0.500 mm, 0.212 mm, 0.106 mm and 0.050 mm), the process was repeated 5 times (Brundrett, 2008). The material was mixed with sucrose and centrifuged at 2000 rpm, spores and spore clusters were transferred into Petri dishes, counted and analyzed with the aid of a stereomicroscope. The morphological characteristics to identify the microorganisms were shape, size, colour, presence of structures like sporiferous saccule and subtending hypha. The genera was assigned according to Morton and Benny (1990) and the guidelines of INVAM. The spore abundance was expressed as the number of AMF spores per gram soil.

2.2. Composting of sewage sludge, food industry waste and maize straw

The experimental system consisted of piles prepared with the sewage sludge from two wastewater treatment plants: 4 tons from a bottling plant and 2 from a paper company, 1 ton of organic waste from a chile packing company and 350 kg of maize straw.

The piles were mixed weekly and watered to provide them with the necessary humidity, during 4 months. The pH, electric conductivity (EC), total Nitrogen and Phosphorous were evaluated according to the norm NOM-021-SEMARNAT-2000, the ratio C/N according to norm NMX-AA-67-1985 and phytotoxicity, according to Zucconi (1981). After the analyses the compost was sieved and stored in a dry place at room temperature.

2.3. Treatments tested

Before the application of the fertilizers, the soil was sterilized 3 times in autoclave (121°C/1 atm, 60 min). Three different biofertilizers were applied to soil (Table 1) to evaluate their influence in the growth of faba bean:

Treatment 1, mixture of 3 kg of soil and 14 g of arbuscular mycorrhizal fungi spores (AMF); Treatment 2, mixture of 3 kg of soil, 1 kg of compost and 14 g of AMF spores (COM/AMF); Treatment 3, mixture of 3 kg of soil and 1 kg of compost (COM); and, control, 4 kg of soil without any treatment. Twenty-eight pots, with two plants of broad been in each pot, were used to evaluate each treatment. The experiment was repeated twice.

Treatment	Soil	Non sterilized soil	COM	AMF
1) AMF	3 kg			14 g
2) AMF/COM	3 kg		1 kg	14 g
3) COM	3 kg		1 kg	
Control		4 kg		

Table 1: Biofertilizers applied to faba bean

AMF: arbuscular mycorrhizal fungi COM: compost

2.4. Evaluation of biofertilizers AMF, AMF/COM and COM over the growth of faba bean

To evaluate the effect of each biofertilizer on the growth of broad bean, the height of the plant, biomasses of the fresh and dried aerial parts and root were recorded separately, after 37, 66 and 100 days. The height of the foliage was measured from the hypocotyl to the upper end of the last leaf. The biomass weight of the fresh foliage was determined from the epicotyl to the upper part of the last shoot. The foliage, in a paper bag, was oven dried (at 65°C until constant weight,) and the dry weight was recorded. The soil of the fresh root was discharged and the root was weighted.

2.5. Statistical analyses

All statistical analyses were done using STATISTICA 7, version for WINDOWS Vista 2006. Differences of means were tested using an analysis of variance (ANOVA). Means were considered to be different when P < 0.05 using the Tukey test.

3. Results and Discussion

3.1. Isolation of AMF

The spores were isolated from soil located in Altzayanca, Tlaxcala, in the central Mexican Altiplano at 2600 m, between 19 ° 26 ' N latitude and 97 ° 48' W longitude. From 100 g of soil were obtained, approximately, 14 g of sieve with an average of 800 spores, only were considered healthy spores, with complete wall, bright and with lipidic granules. The AMF identified in the soil, belong to the genera *Acaulospora* (Acaulosporaceae), *Glomus* and *Sclerocystis* (Glomeraceae), *Scutellospora* and *Gigaspora* (Gigasporaceae), all of them of the class Glomeromycetes; the most abundant was *Glomus* (Schüßler& Walker, 2010).

This same genus has been reported as the most abundant in studies carried out in several countries and with different cultivars, for example in soils cultivated with two varieties of corn and added with green material and manure (Serralde & Ramirez, 2004), in wheat under different tillage systems (Schalamuk et al., 2007); in subtropical agricultural land planted with vegetables and fruit (Wang et al., 2008); has also been found in regions of Pakistan, associated with the rhizosphere of *Menthalongifolia* and *Menthaarvensis* (Burni et al., 2011) and in the Himalayan region associated with medicinal plants (*Catharanthusroseus, Ocimum* spp and *Asparagus racemosus*) (Gaur & Kaushik, 2012).

3.2. Physicochemical characterization and phytotoxicity of compost

The mixture to prepare the compost was prepared with 6 tons of sewage sludge, 1 ton of chile waste and 350 kg of maize straw, it had an initial ratio 2000). C/N of 30/1, recommended obtaining good quality compost (Catellanos et al., After 4 months it was obtained a mature compost that was no phytotoxic to lettuce seeds according to Zucconi (1989). The physical and chemical properties of the compost are in Table 2 and meet the Mexican standards values (NTEA-006-SMA-RS-2006), for the production of fertilizers from organic waste.

The norm NTEA-006-SMA-RS-2006 does not specify the maximum value of N, but this element was measured because it is one of the main factors limiting plant growth (Myrold, 1999). Besides compost not only provides N, but also P that is important for plant nutrition (Saidiet at., 2009) and the value in the compost meets the limits. P is important in the biosynthesis of phospholipids and nucleic acids, also plays a major role during flowering and fructification of plants (Calleja-Cervantes et al., 2015).

	Compost	Soil	Maximum permited	Reference
N (mg/kg)	13.21	4.65		
P (%)	0.167	0.42	> 0.1	NTEA-006-SMA-RS-2006
C/N	16.46	9.13	< 20/1	Castellanos, 2000
рН	7.23	7.34	6.5 – 8.0	NTEA-006-SMA-RS-2006
EC (dS/m)	1.12	0.04	< 2	IUSS (2007)

Table 2: Physicochemical properties of the compost

The pH is another important physicochemical parameter of compost, that impacts significantly in the availability and assimilation of essential nutrients for plants, such as the N, P, Fe, Mn, Cu and Zn; for example the pH range favoring the availability of N is 6 to 8.5, while for fixing P is between 6 and 7.

In addition, the pH has a strong influence in determining the proliferation and activity of many soil microorganisms (Sparks, 2008). The pH of the compost produced in this work was 7.23, which is suitable for the availability of nutrients and is in the range indicated in the standard (NTEA-006-SMA-RS-2006) (Table 2). The pH value coincides with that obtained in other studies, the pH of a compost made from waste sludge and fresh plants was 7.2 (Jouraiphy et al., 2005) and for a compost prepared with sludge and sawdust was 7.39 (Cai et al., 2010).

The electrical conductivity (EC) indicates the presence of soluble salts, is related to the concentration of Na⁺, Ca⁺², K⁺, Mg⁺², Cl⁻ and SO₄⁻², high concentrations of these ions can directly affect crop development, however this will depend on the tolerance of the crop to salinity, soil type and irrigation practices employed (McMahon &Valdés, 2011). Soil salinity is one of the main limiting factors of fertility; the recommended EC value is $\leq 1.50 \text{ dS/m}$, above this value the morphological, physical and chemical soil properties are strongly modified, and the salinity could be toxic to plants (Serralde & Ramírez, 2004). The EC of compost prepared is in the suggested limit (Table 2), so its application to crops could not be toxic the determined value is similar to that reported by other authors, Tongetti et al. (2007) reported a CE of 1.6 dS/m for compost obtained from garbage.

3.3. Influence of biofertilizers AMF, AMF/COM and COM on faba bean growth

Bio-fertilizers such as compost and arbuscular mycorrhizal fungi have been used to improve agricultural production. To determine the effect of the biofertilizers in the growth of faba bean compared with soil, samples of the faba bean plants were analyzed at three growth stages, 33, 66 and 100 days, to assess the height of the foliage, fresh and dry foliageand root biomass weight.

The results of height and biomass of faba bean, obtained with the tree biofertilizers and the control were compared, and it was observed that the parameters did not show significant differences among treatments and control after 66 days of cultivation. The differences in the height and biomass of faba bean, under the different treatments, were evident after 100 days of cultivation (Table 3).

Parameter	Control	Treatment 1 (AMF)	Treatment2 (AMF/COM)	Treatment3 (COM)
Height (cm)	77.28 (b)	87.42 (a)	88.42 (a)	74.42 (c)
Freshfoliage (g)	39.90 (b)	55.64 (a)	55.54 (a)	50.38 (ab)
Dryfoliage (g)	6.48 (b)	8.53 (ab)	9.11 (a)	7.60 (ab)
Fresh root biomass (g)	20.3 (b)	41.5 (a)	29.2 (ab)	24.4 (b)

Table 3: Average values of biomass of faba bean after 100 days of cultivation	in
he presence of biofertilizers	

Equal letters in rows indicate no significant difference

From results in table 3, it can be observed that the application of AMF with or without compost, increased the plant height, fresh and dry biomass of foliage and fresh root biomass of faba bean, compared to the use of compost or soil. The symbiosis with AMF had a positive effect on the vegetative growth of the plants, because AMF increase the surface area of absorption, improving the assimilation of nutrients, thus benefiting the nutritional status of the plant (Joner et al., 2000; Rubio et al., 2003). The mycorrhizal inoculum improved the uptake of soil nutrients or those provided by the compost, the faba bean height, production of foliage and root was improved.

The fresh foliage biomass of faba bean cultivated in the presence of AMF, with or without compost (Treatments 1 and 2), had an increase of 39% compared to control, this is an important result because, the number of leaves and leaf area are very important characteristics for plant development.

Leaves are the photosynthetic machinery in plants, so it is expected that a larger leaf area will provide more carbohydrates and a stronger plant. The AMF contributed positively to the radical development (Table 3). However, the root development in plants cultivated with AMF/COM was lower than when adding only the AMF, possibly because some elements in compost inhibited accurate colonization of AMF, then the positive effect of AMF is diminished, as noticed Eghball et al. (2004). The balance of fresh and root biomass is an indicator that the plant was developed successfully. The application of compost also improved the foliage biomass, but it was more effective the application of AMF; the lowest parameters of faba bean were recorded in the control plants.

All these results are consistent with those observed by other authors, for example *Euphorbia prostrata* significantly increased fresh weight and root biomass with AMF inoculation and vermicompost (Channashettar et al., 2008). The weight of corn plants grown in peat, inoculated with *G. fasciculatum*, vermicompost, and with bacteria was considerably increased (Gutiérrez et al., 2008).

4. Conclusions

The application of biofertilizers, such as compost and AMF are a viable alternative to the use of chemical fertilizers, so it is necessary to promote their use. The composting of sewage sludge, food and agricultural waste resulted in a bio economical way to reduce contamination, obtain an added value product that helps to improve nutritional properties of farm. It is very important to note that the use of soil microorganisms, such as AMF; compost and the mixture of AMF and compost are a sustainable option the use of chemical fertilizers.

Furthermore, it is very important to analyze the physical and chemical properties of biofertilizers, to ensure they comply with regulations to be used in farm soils. In this study, the composting of the sludge from two water treatment plants, waste from a food company and straw corn, produced a compost suitable for the fertilization of soil and enhanced the productivity of faba bean. This is important because it is an environmental and economic way to dispose of these types of waste, is obtained a product with an economical value and can be used to improve the properties of soil.

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