



The colossal influence of biological fertilization on medicinal and aromatic plants

Syed Arif Hussain¹, Khalil Ahmad¹, Arif-un-Nisa Naqvi¹, Tika Khan¹, Mehsoor Ahmed Nafees¹, Maqsood Hussain², Qamar Abass¹, Muhammad Ali¹, MohammadAkber³, Sher Wali Khan¹

¹*Department of Biological Sciences, Karakoram International University, Gilgit- Baltistan, Pakistan*

²*Integrated Mountain Research Centre, Karakoram International University, Gilgit-Baltistan 15100, Pakistan*

³*Department of Environmental Sciences, Karakoram International University, Gilgit- Baltistan, Pakistan*

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Abstract

The need of increase food production in the most of developing countries becomes an ultimate goal to meet the dramatic expansion of their population. However, this is also associated many cases with a reduction of the areas of arable land which leaves no opinion for farmers but to increase the yield per unit area through the use of improved the crop varieties, irrigation and fertilization. The major problem facing the farmer is that he cannot afford the cost of these goods, particularly that of chemical fertilizers. Moreover, in countries where fertilizer production relies on imported raw materials, the costs are even higher for farmer and for the country. Besides this, chemical fertilizers production and utilization are considered as air, soil and water polluting operations. The utilization of bio-fertilizers is considered today by many scientists as a promising alternative, particularly for developing countries. Bio-fertilization is generally based on altering the rhizosphere flora, by seed or soil inoculation with certain organisms, capable of inducing beneficial effects on a compatible host. Bio-fertilizers mainly comprise nitrogen fixes, phosphate dissolvers or vesicular-arbuscular mycorrhizas and silicate bacteria. Growth characters, yield, essential oil and its constituents, fixed oil, carbohydrates, soluble sugars and nutrients contents of medicinal plants were significantly affected by adding the biological fertilizers compared with recommended chemical fertilizers.

*Corresponding Author: Syed Arif Hussain ✉ arif.bio@kiu.edu.pk

Introduction

A biological fertilizer (also bio-fertilizer) is a substance which contains living microorganisms, when applied to seed, plant surfaces, or soil colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey 2003). Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. Biofertilizers can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of bio-fertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Since they play several roles, a preferred scientific term for such beneficial bacteria is *plant-growth promoting rhizobacteria* (PGPR). Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. Hence, bio-fertilizers do not contain any chemicals which are harmful to the living soil. *Anabaena* in association with water fern *Azolla* contributes nitrogen up to 60 kg/ha/season and also enriches soils with organic matter (Vessey 2003). Other types of bacteria, so-called phosphate-solubilizing bacteria, such as *Pantoea agglomerans* strain P5 or *Pseudomonas putida* strain P13 are able to solubilize the insoluble phosphate from organic and inorganic phosphate sources (Subba Rao 1984). In fact, due to immobilization of phosphate by mineral ions such as Fe, Al and Ca or organic acids, the rate of available phosphate (Pi) in soil is well below plant needs. In addition, chemical Pi fertilizers are also immobilized in the soil, immediately, so that less than 20 percent of added fertilizer is absorbed by plants. Therefore, reduction in Pi resources, on one hand, and environmental pollutions resulting from both production and applications of chemical Pi fertilizer, on the other hand, have already demanded the use of

new generation of phosphate fertilizers globally known as phosphate solubilizing bacteria or phosphate bio-fertilizers. At present, however, the yield of many crops in the world has reached a plateau. Moreover, the negative effects of heavy applications of chemical inputs are becoming apparent, in terms of both production and the environment, especially in the case of medicinal plants. Physiological disturbance of plant metabolism is common, due to the accumulation of excess plant nutrients in the soil. The spread of soil-borne diseases is a threat to medicinal plants production, especially where monoculture is prevailing. Pollution of underground and surface water by nitrates is sometimes reported from medicinal plants producing areas. Quality deterioration, in terms of a decrease in the content of vitamins, sugars active principals, is becoming a subject of concern. All these factors are giving farmers an interest in the function and utilization of soil microorganisms, as a way of repairing the damage from the overuse of chemical inputs.

Although many microbial materials are sold commercially, most of them are not microbiologically defined, i.e. the microorganisms contained in the products are not identified, and the microbial composition is not fixed. Many of these commercial products are advertised as if they could solve any problem a farmer is likely to encounter. Because most extension advisors lack any knowledge of microbial products, confusion and trouble frequently occur. The main objectives of this manuscript are: (i) Types of biological fertilization, i.e. nitrogen fixation, phosphate solubilizing microorganisms, sulphur oxidizing bacteria, silicate bacteria, plant growth promoting rhizobacteria (PGPR), mycorrhizal fungi, decomposition, and (ii) effect of biological fertilization on medicinal plants. This manuscript concentrates on biological fertilization its effects on medicinal plants because of there are no review article was published before concentrated on these items.

Types of biological fertilization

Nitrogen fixation Nitrogen can be found in many forms in our environment. Nitrogen is also very important for plants to live. The earth's atmosphere is made up of 78 percent nitrogen in the form of a colorless, odorless, nontoxic gas. The same nitrogen gas found in the atmosphere can be found in spaces between soil particles. However, plants are unable to use this form of nitrogen. Certain microorganisms found in the soil are able to convert atmospheric nitrogen into forms plants can use. This is called biological nitrogen fixation (Subba Rao 1984). One of the most interesting forms of biological nitrogen fixation is that which takes place by microorganisms living in very small nodules on the roots of certain plants such as legumes.

This is called symbiotic nitrogen fixation. A symbiotic relationship is an association or relationship where both organisms mutually benefit. In this case, microorganisms obtain food and energy from the root of the plant while producing nitrogen the plant can use for growth and development. The form of nitrogen produced is the same form of nitrogen that is found in several types of commercial nitrogen fertilizers (Subba Rao 1984). The microorganism's ability to fix atmospheric nitrogen is often discussed in terms of the plant's ability to fix nitrogen. The amount of fixation that takes place is strongly influenced by soil conditions. Factors such as moisture, temperature, oxygen supply and fertility in the soil can influence fixation. Diseases and insects can also affect the degree of nitrogen fixation (Subba Rao 1984).

One of the most common groups of plants that fix nitrogen is legumes. Of the total nitrogen required by legumes, generally about half is nitrogen fixed from the atmosphere, with the remainder being taken up from residual nitrate in the soil. This means that where legumes are grown, outside applications of manure or fertilizer nitrogen are not needed. Different legumes also vary in the amount of total nitrogen they can fix (Subba Rao 1984).

Phosphate Solubilizing Microorganisms

Phosphorous is added to cultivated soil in different forms as mineral phosphate fertilizers or organic manure, the soluble P in these fertilizers is quickly turns into unavailable form for plant nutrition, this problem is well known in Egyptian soils specially those rich in Calcium Carbonate (El-Gamal 1996), phosphorous is commonly deficient in most natural soils, since it is fixed as calcium phosphates in alkaline soils (Cunningham and Kuiuack 1992; Goldstein 1995). Faisal and El-Dawwy 1999 indicated that inadequate phosphorous is a widespread problem in crop production in Egypt and elsewhere. In Egypt's alkaline soils, low solubility Calcium triphosphate are formed following the application of P fertilizer, soluble phosphate ions also are adsorbed on solid calcium carbonate surfaces. Fortunately, soil microorganisms known as phosphate solubilizing microorganisms play a fundamental role in converting P fixed form to be soluble ready available for plant nutrition. The microbial breakdown of soil organic matter is associated with an increase organic, inorganic acids and CO₂ production with possibly increases the solubility of soil phosphate (Quastel 1965, Taha *et al.*, 1969, Mishustin *et al.*, 1972, Alexander 1977, Pamela and Hayasaka 1982, Subba Rao 1984 and Curl and Truelove 1985). However Cunningham and Kuiuack 1992 and Goldstein 1995 reported that, insoluble calcium phosphate can be dissolved and made available to plants by rhizosphere microorganisms via a mechanism that thought to involve the release of organic acids. Most studies of phosphate solubilizing microorganisms involve inoculating the soils (Mishustin *et al.*, 1972). Inoculation with phosphate-dissolvers is claimed to increase the yield of many agriculture crops (Taha *et al.*, 1969; Ewada 1976; Ocampo *et al.*, 1978; Guar *et al.*, 1980; Abdel-Nasser *et al.*, 1982; Subba Rao 1984). Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate (Goldstein 1986). Among the bacterial genera with this capacity are *Pseudomonas*, *Bacillus*,

Rhizobium, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aereobacter*, *Flavobacterium* and *Erwinia*. There are considerable populations of phosphate-solubilizing bacteria in soil and in plant rhizospheres (Sperberg 1958; Katznelson *et al.*, 1962; Raghu and MacRae, 1962; Alexander 1977). These include both aerobic and anaerobic strains, with a prevalence of aerobic strains in submerged soils (Raghu and MacRae 1962). A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere in comparison with nonrhizosphere soil (Katznelson *et al.*, 1952; Raghu and Mac Rae, 1962).

Sulphur oxidizing bacteria

Sulphur is one of the essential plant nutrients and it contributes to yield and quality of crops. Sulphur occurs in a wide variety of organic and inorganic combinations. The transfer of sulphur between the inorganic and organic pool is entirely caused by the activity of the soil biota, particularly the soil microbial biomass, which has greatest potential for both mineralization and also for subsequent transformation of the oxidation state of sulphur. *Thiobacilli* play an important role in sulphur oxidation in soil. Sulphur oxidation is the most important step of sulphur cycle, which improves soil fertility. It results in the formation of sulphate, which can be used by the plants, while the acidity produced by oxidation helps to solubilize plant nutrients and improves alkali soils (Wainwright 1984). The sulphur oxidizing microorganisms are primarily the gram negative bacteria currently classified as species of *Thiobacillus*, *Thiomicrospira* and *Thiosphaera*, but heterotrophs, such as some species of *Paracoccus*, *Xanthobacter*, *Alcaligenes* and *Pseudomonas* can also exhibit chemolithotrophic growth on inorganic sulphur compounds (Kuenen and Beudeker 1982). Two clear metabolic types exist in this group: The obligate chemolithotrophs, which can only grow when supplied with oxidizable sulphur compounds (and CO₂ as the source of metabolic carbon) and heterotrophs that can also use the chemolithoautotrophic mode of growth. The obligate

chemolithotrophs include *Thiobacillus thioparus*, *T. neapolitanus*, *T. denitrificans* (facultative denitrifier), *Thiobacillus thiooxidans* (extreme acidophile), *Thiobacillus ferrooxidans* (acidophilic ferrous ironoxidizer), *Thiobacillus halophilus* (halophile) and some species of *Thiomicrospira*. The heterotrophs include *Thiobacillus novellus*, *T. acidophilus* (acidophile), *Thiobacillus aquaesulis* (moderate thermophile), *Thiobacillus intermedius*, *Paracoccus denitrificans*, *P. versutus* *Xanthobacter tagetidis*, *Thiosphaera pantotroph* and *Thiomicrospira thyasirae*. Several *Thiobacillus* species are able to utilize mixtures of inorganic and organic compounds simultaneously, often referred to as mixotrophic growth (Kuenen and Beudeker 1982). Depending on the ratio of inorganic and organic substrates, CO₂ may serve as an additional carbon source. Of the 13 species of the genus *Thiobacillus* recognized, occurring in diverse habitats, only five species are important in sulphur oxidation in soil (Starkey 1966). Four of these *Thiobacillus thiooxidans*, *T. ferrooxidans*, *T. thioparus* and *T. denitrificans* are obligate chemoautotrophs while *T. novellus* is considered a facultative chemoautotroph (Taylor and Hoare 1969). Also fungi are capable of oxidizing elemental sulphur and thiosulphate, which include, *Alternaria tenuis*, *Aureobasidium pullulans*, *Epicoccum nigrum*, a range of *Penicillium* species (Wainwright 1984), *Scolecobasidium constrictum* *Myrothecium cinctum* and *Aspergillus* (Shinde *et al.*, 1996). Though sulphur traces may be oxidized in soil by various species of microorganisms and fungi, Waksman (1932) pointed out *Thiobacilli* as the most characteristic group of microorganism performing the oxidative part of sulphur transformation in soil. Studies on the distribution of *Thiobacillus thiooxidans* and *T. thioparus* showed that these bacteria are found in an active state mainly in soils fertilized with sulphur. The distribution of *T. thioparus* in soils was studied by Starkey (1934) who demonstrated the almost ubiquitous presence of bacteria in alkaline and neutral soils and their absence in strongly acid soils fertilized with sulphur. The widespread occurrence of *Thiobacilli* in soils fertilized with sulphur or in soils in

which accumulation of sulphur compounds occurs under natural conditions (marshes and peats) indicates that these bacteria play an important role in the oxidation of sulphur and its compounds in soils. Inoculation of *Thiobacilli* generally increases the rate of sulphur oxidation (Kapoor, and Mishra 1989). Kapoor, and Mishra (1989) observed that sulphur was rapidly oxidized in a field soil of pH 8.0 and the rate of oxidation could be enhanced by inoculation with *T. thiooxidans*.

Silicate bacteria

Silicate bacteria or biological potassium fertilizer can activate the fixed potassium for plant nutrition, as well as prevention and control of plant diseases; also it can effectively prevent crops from early aging and have a strong resistance to drought and cold (Subba Rao 1984). Zahra *et al.*, (1984) reported that, silicatedissolving bacteria played a pronounced role in the biological weathering of soil minerals and it can promote K and Si releasing from feldspar. Sheng *et al.*, 2003 showed that, silicate-dissolving bacteria could activate soil P, K, and micronutrients reserves and promote plant growth. Styriakova *et al.*, 2003 reported that, the activity of silicate dissolving played a pronounced role in release of Si, Fe and K from feldspar and Fe oxyhydroxides.

Plant growth promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) comprise a diverse group of rhizosphere-colonizing bacteria and diazotrophic microorganisms which, when grown in association with a plant, stimulate growth of the host. PGPR can affect plant growth and development indirectly or directly (Glick 1995; Vessey 2003). In indirect promotion, the bacteria decrease or eliminate certain deleterious effects of a pathogenic organism through various mechanisms, including induction of host resistance to the pathogen (Van Loon 2007). direct promotion, the bacteria may provide the host plant with synthesized compounds; facilitate uptake of nutrients; fix atmospheric nitrogen; solubilize minerals such as phosphorus; produce siderophores, which solubilize and sequester

iron; synthesize phytohormones, including auxins, cytokinins, and gibberellins, which enhance various stages of plant growth; or synthesize enzymes that modulate plantgrowth and development (Lucy *et al.*, 2004; Gray and Smith 2005). radyrhizobium and Sinorhizobium, of the bacterial family Rhizobiaceae, are known for their ability to fix atmospheric nitrogen while living symbiotically on and nodulating the roots of leguminous plants. However, members of this family also display non-specific associative interactions with roots of other plants, without forming nodules (Van Loon 2007). These rhizobial strains are presumed to produce plant growth regulators, and are classified as PGPR (Vessey 2003).

Mycorrhizal fungi

Mycorrhizal types Of the many types of mycorrhizal association (Harley and Smith 1983), two are of major economic and ecological importance: ectomycorrhizal associations, and the endomycorrhizal association of the vesicular-arbuscular (VA) type. In ectomycorrhizal associations, the fungi invade the cortical region of the host root without penetrating cortical cells. The main diagnostic features of this type of mycorrhiza are (i) the formation within the root of a hyphal network known as the Hartig net around cortical cells and (ii) a thick layer of hyphal mat on the root surface known as *sheath* or *mantle*, which covers feeder roots. Infection of host plants by ectomycorrhizal fungi often leads to changes in feeder roots that are visible to the naked eye. Feeder roots colonized by the fungi are thicker and more branched than uncolonized roots; ectomycorrhizal feeder roots also tend to be colored differently. In endomycorrhizal associations of the VA type, the fungi penetrate the cortical cells and form clusters of finely divided hyphae known as *arbuscules* in the cortex. They also form vesicles, which are membranebound organelles of varying shapes, inside or outside the cortical cells. Arbuscules are believed to be the sites where materials are exchanged between the host plant and the fungi. Vesicles generally serve as storage structures, and when they are old they can serve as reproductive structures. Vesicles and

arbuscules, together with large spores, constitute the diagnostic features of the VA mycorrhizas. Roots have to be cleared and stained in specific ways and examined under a microscope to see that they are colonized by VA mycorrhizal fungi. Because vesicles are not always found in these types of mycorrhizal associations, some researchers now prefer the designation arbuscular mycorrhiza (AM) over the term vesicular-arbuscular (VA) mycorrhiza. Both AM fungi and ectomycorrhizal fungi extend hyphae from the root into the soil, and these external (or extraradical) hyphae are responsible for translocating nutrients from the soil to the root. Most ectomycorrhizal fungi belong to several genera within the class basidiomycetes, while some belong to the zygosporic zygomycetes and ascomycetes. On the other hand, AM fungi belong to six genera within the azygosporous zygomycetes.

Host specificity

AM associations occur in a wide spectrum of tropical and temperate tree species. They are known not to occur only in a few plants, namely members of the families Amaranthaceae, Pinaceae, Betulaceae, Cruciferae, Chenopodiaceae, Cyperaceae, Juncaceae, Proteaceae, and Polygonaceae. The ectomycorrhizas, on the other hand, occur primarily in temperate forest species, although they have been reported to colonize a limited number of tropical tree species.

Functions of mycorrhizal fungi

Results of experiments suggest that AM fungi absorb N, P, K, Ca, S, Cu, and Zn from the soil and translocate them to associated plants (Tinker and Gildon 1983). However, the most prominent and consistent nutritional effect of AM fungi is in the improved uptake of immobile nutrients, particularly P, Cu, and Zn (Pacovsky 1986; Manjunath and Habte 1988). The fungi enhance immobile nutrient uptake by increasing the absorptive surfaces of the root. The supply of immobile nutrients to roots is largely determined by the rate of diffusion. In soils not adequately supplied with nutrients, uptake of nutrients by plants far exceeds the rate at which the

nutrients diffuse into the root zone, resulting in a zone around the roots depleted of the nutrients. Mycorrhizal fungi help overcome this problem by extending their external hyphae to areas of soil beyond the depletion zone, thereby exploring a greater volume of the soil than is accessible to the unaided root. Enhanced nutrient uptake by AM fungi is often associated with dramatic increase in dry matter yield, typically amounting to several-fold increases for plant species having high dependency on mycorrhiza. AM fungi may have biochemical capabilities for increasing the supply of available P and other immobile nutrients. These capabilities may involve increases in root phosphatase activity, excretion of chelating agents, and rhizosphere acidification. However, these mechanisms do not appear to explain the very pronounced effect the fungi have on plant growth (Habte and Manjunth 1991). AM fungi are often implicated in functions which may or may not be related to enhance nutrient uptake. For example, they have been associated with enhanced chlorophyll levels in leaves and improved plant tolerance of diseases, parasites; water stress, salinity, and heavy metal toxicity (Bethlenfalvay and Gabor 1992). Moreover, there is increasing evidence that hyphal networks of AM fungi contribute significantly to the development of soil aggregates, and hence to soil conservation (Miller and Jastrow 1992).

Charcoal application

The amount of nutrients (N, P, and K) absorbed by the shoots showed a trend similar to that of the shoot fresh weight. The amount of N fixed by the nodules and transported to the shoots was calculated by subtracting the N content of the shoots of the plants not inoculated with rhizobia from the N content of the inoculated plants. The addition of charcoal increased this amount of N 2.8-4.0 times. Added charcoal also increased the nodule weight by 2.3 times. Significant correlation was observed between the increments of P and N, suggesting that the stimulation of nitrogen fixation by charcoal addition may be due to the stimulation of P uptake. Charcoal may stimulate the growth of AMF by the following mechanism. Charcoal

particles have a large number of continuous pores with a diameter of more than 100 μ m. They do not contain any organic nutrients, because of the carbonization process. The large pores in the charcoal may offer a new microhabitat to the AMF, which can obtain organic nutrients through mycelia extended from roots. This may enable the AMF to extend their mycelia far out from the roots, thus collecting a larger amount of available phosphate (Nishio and Okano 1991).

Decomposition

Organic matter decomposition serves two functions for the microorganisms, providing energy for growth and supplying carbon for the formation of new cells. Soil organic matter (SOM) is composed of the “*living*” (microorganisms), the “*dead*” (fresh residues), and the “*very dead*” (humus) fractions. The “*very dead*” or humus is the long-term SOM fraction that is thousands of years old and is resistant to decomposition. Soil organic matter has two components called the active (35%) and the passive (65%) SOM. Active SOM is composed of the “*living*” and “*dead*” fresh plant or animal material which is food for microbes and is composed of easily digested sugars and proteins. The passive SOM is resistant to decomposition by microbes and is higher in lignin. Microbes need regular supplies of active SOM in the soil to survive in the soil. Long-term no-tilled soils have significantly greater levels of microbes, more active carbon, more SOM, and more stored carbon than conventional tilled soils. A majority of the microbes in the soil exist under starvation conditions and thus they tend to be in a dormant state, especially in tilled soils. Dead plant residues and plant nutrients become food for the microbes in the soil. Soil organic matter (SOM) is basically all the organic substances (anything with carbon) in the soil, both living and dead. SOM includes plants, blue green algae, microorganisms (bacteria, fungi, protozoa, nematodes, beetles, springtails, etc.) and the fresh and decomposing organic matter from plants, animals, and microorganisms (Hoorman and Islam 2010).

Climate, Temperature, and pH effects on SOM

SOM is affected by climate and temperature. Microbial populations double with every 10 degree Fahrenheit change in temperature. If we compare the tropics to colder arctic regions, we find most of the carbon is tied up in trees and vegetation above ground. In the tropics, the topsoil has very little SOM because high temperatures and moisture quickly decompose SOM. Moving north or south from the equator, SOM increases in the soil. The tundra near the Arctic Circle has a large amount of SOM because of cold temperatures. Freezing temperatures change the soil so that more SOM is decomposed than in soils not subject to freezing. Moisture, pH, soil depth, and particle size affect SOM decomposition. Hot, humid regions store less organic carbon in the soil than dry, cold regions due to increased microbial decomposition. The rate of SOM decomposition increases when the soil is exposed to cycles of drying and wetting compared to soils that are continuously wet or dry. Other factors being equal, soils that are neutral to slightly alkaline in pH decompose SOM quicker than acid soils; therefore, liming the soil enhances SOM decomposition and carbon dioxide evolution. Decomposition is also greatest near the soil surface where the highest concentration of plant residues occur. At greater depths there is less SOM decomposition, which parallels a drop in organic carbon levels due to less plant residues. Small particle sizes are more readily degraded by soil microbes than large particles because the overall surface area is larger with small particles so that the microbes can attack the residue. A difference in soil formation also occurs traveling east to west across the United States. In the east, hardwood forests dominated and tree tap roots were high in lignin, and deciduous trees left large amounts of leaf litter on the soil surface. Hardwood tree roots do not turn over quickly so organic matter levels in the subsoil are fairly low. In forest soils, most of the SOM is distributed in the top few inches. As you move west, tall grassland prairies dominated the landscape and topsoil formed from deep fibrous grass root systems. Fifty percent of a grass root dies and is replaced every year and grass

roots are high in sugars and protein (higher active organic matter) and lower in lignin. So soils that formed under tall grass prairies are high in SOM throughout the soil profile. These prime soils are highly productive because they have higher percentage of SOM (especially active carbon), hold more nutrients, contain more microbes, and have better soil structure due to larger fungal populations.

Carbon to Nitrogen(C:N) ratio Low nitrogen content or a wide C:N ratio is associated with slow SOM decay. Immature or young plants have higher nitrogen content, lower C:N ratios and faster SOM decay. For good composting, a C:N ratio less than 20 allows the organic materials to decompose quickly (4 to 8 weeks) while a C:N ratio greater than 20 requires additional N and slows down decomposition. The C:N ratio of most soils is around 10:1 indicating that N is available to the plant. The C:N ratio of most plant residues tends to decrease with time as the SOM decays. This results from the gaseous loss of carbon dioxide. Therefore, the percentage of nitrogen in the residual SOM rises as decomposition progresses. The 10:1 C:N ratio of most soils reflects an equilibrium value associated with most soil microbes (bacteria 3:1 to 10:1, fungus 10:1 C:N ratio). Bacteria are the first microbes to digest new organic plant and animal residues in the soil. Bacteria typically can reproduce in 30 minutes and have high N content in their cells (3 to 10 carbon atoms to 1 nitrogen atom or 10 to 30% nitrogen). Under the right conditions of heat, moisture, and a food source, they can reproduce very quickly. Bacteria are generally less efficient at converting organic carbon to new cells. Aerobic bacteria assimilate about 5 to 10 percent of the carbon while anaerobic bacteria only assimilate 2 to 5 percent, leaving behind many waste carbon compounds and inefficiently using energy stored in the SOM. Fungus generally release less carbon dioxide into the atmosphere and are more efficient at converting carbon to form new cells. The fungus generally captures more energy from the SOM as they decompose it, assimilating 40 to 55 percent of the carbon. Most fungi consume organic matter higher in cellulose and lignin, which is slower and tougher to

decompose. The lignin content of most plant residues may be of greater importance in predicting decomposition velocity than the C:N ratio.

Mycorrhizal fungi live in the soil on the surface of or within plant roots. The fungi have a large surface area and help in the transport of mineral nutrients and water to the plants. The fungus life cycle is more complex and longer than bacteria. Fungi are not as hardy as bacteria, requiring a more constant source of food. Fungi population levels tend to decline with conventional tillage. Fungi have a higher carbon to nitrogen ratio (10:1 carbon to nitrogen or 10% nitrogen) but are more efficient at converting carbon to soil organic matter. With high C:N organic residues, bacteria and fungus take nitrogen out of the soil. Protozoa and nematodes consume other microbes. Protozoa can reproduce in 6-8 hours while nematodes take from 3 days to 3 years with an average of 30 days to reproduce. After the protozoa and nematodes consume the bacteria or other microbes (which are high in nitrogen), they release nitrogen in the form of ammonia. Ammonia (NH_4^+) and soil nitrates (NO_3^-) are easily converted back and forth in the soil. Plants absorb ammonia and soil nitrates for food with the help of the fungi mycorrhizal network. Microorganism populations change rapidly in the soil as SOM products are added, consumed, and recycled. The amount, the type, and availability of the organic matter will determine the microbial population and how it evolves. Each individual organism (bacteria, fungus, and protozoa) has certain enzymes and complex chemical reactions that help that organism assimilate carbon. As waste products are generated and the original organic residues are decomposed, new microorganisms may take over, feeding on the waste products, the new flourishing microbial community (generally bacteria), or the more resistant SOM. The early decomposers generally attack the easily digested sugars and proteins followed by microorganisms that attack the more resistant residues. It can be summarized that (i) Microorganisms abound in the soil and are critical to decomposing organic residues and recycling soil

nutrients. Bacteria are the smallest and most hardy microbe in the soil and can survive under harsh conditions like tillage. Bacteria are only 20-30% efficient at recycling carbon, have high nitrogen content (3 to 10 carbon atoms to nitrogen atom or 10 to 30% nitrogen), lower carbon content, and a short life span. Carbon use efficiency is 40-55% for mycorrhizal fungi so they store and recycle more carbon (10:1 carbon to nitrogen ratio) and less nitrogen (10%) in their cells than bacteria. Fungi are more specialized but need a constant food source and grow better under no-till conditions. (ii) Soil organic matter (SOM) is composed of the "living" (microorganisms), the "dead" (fresh residues), and the "very dead" (humus) fractions. Active SOM is composed of the fresh plant or animal material which is food for microbes and is composed of easily digested sugars and proteins. The passive SOM is resistant to decomposition by microbes (higher in lignin). Active SOM improves soil structure and holds plant available nutrients. Every 1% SOM contains 1,000 pounds of nitrogen, 100 pounds of phosphorus, 100 pounds of potassium, and 100 pounds of sulfur along with other essential plant nutrients. Tillage destroys SOM by oxidizing the SOM, allowing bacteria and other microbes to quickly decompose organic residues. Higher temperatures and moisture increase the destruction of SOM by increasing microbial populations in the soil. Organic residues with a low carbon to nitrogen (C:N) ratio (less than 20) are easily decomposed and nutrients are quickly released (4 to 8 weeks), while organic residue with a high C:N ratio (greater than 20) decompose slowly and the microbes will tie up soil nitrogen to decompose the residues. Protozoa and nematodes consume other microbes in the soil and release the nitrogen as ammonia, which becomes available to other microorganisms or is absorbed by plant roots.

Effect of biological fertilization on medicinal plants

Growth characters of rosemary, K, N content essential oil constituents (alpha-pinene, B-pinene, limonene, 1,8- cineole, linalool, camphor, B-terpineol, borneol, terpinen 4-ol, carvone, thymol, carvacrol,

linalylacetate, geranylacetate, B-caryophyllene, caryophyllene oxide) were significantly increased under biofertilizer (*Azotobacter vinelandii*) treatments (Leithy and El-Meseiry 2006). The effects of biofertilization on growth, fruit yield, and oil composition of fennel plants were investigated by Mahfouz and Sharaf- Eldin (2007). Application of biofertilizer, which was a mixture of *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Bacillus megatherium* applied with chemical fertilizers (only 50% of the recommended dosage of NPK) increased vegetative growth (plant height, number of branches, and herb fresh and dry weight per plant) compared to chemical fertilizer treatments only. The tallest plants, the highest number of branches per plant, and the highest fresh and dry weights of plants were obtained from the treatment of biofertilizer plus a half dose of chemical fertilizer (357 kg ammonium sulphate + 238 kg calcium super phosphate + 60 kg potassium sulphate ha⁻¹). The lowest fresh and dry weights of plants occurred with the 50% NPK. Also, addition of biofertilizer with the chemical fertilizer increased these characters more than the half dose of chemical fertilizer alone. Total carbohydrates in the dry plant material were influenced by the biofertilizer. The highest values of total carbohydrates were found in the treatment with biofertilizer plus a half dose of nitrogen and phosphorus. Nitrogen, phosphorus, and potassium levels in the plant tissue increased when soil was inoculated by nitrogen-fixing bacteria, phosphate dissolving bacteria, and a mixture of all strains, respectively. The least amount of N, P and K in the plant tissue occurred with the half dose of chemical fertilizer. Essential oil content in the fennel fruits was increased due to inoculation compared to the half dose of chemical fertilizer. The highest oil yield per plant was observed with the treatment of biofertilizer plus a half dose of nitrogen and phosphorus. The lowest amount of essential oil yield was obtained with the half dose of chemical fertilizer. Oxygenated compounds were increased as a result of using biofertilizer. The highest anethol (*trans*-1-methoxy- 4-(prop-1-enyl) benzene; C₁₀H₁₂O) in fennel essential oil occurred with the half dose of N,

P, and K and inoculation with *Bacillus megatherium*. The effect of compost and biofertilizers on the growth, yield and essential oil constituents of marjoram (*Majorana hortensis* L.) was investigated by Gharib *et al.*, (2008). Forty five days old seedling were transplanted in soil treated with 15 and 30% aqueous extracts of compost and/or biofertilizers (mixture of *Azospirillum brasilienses*, *Azotobacter chroococcum*, *Bacillus polymyxa* and *B. circulans*) in addition to the recommended nitrogen, phosphorus and potassium (NPK) doses as control. Use of combined treatment of bio-fertilizers gave better results for all studied traits than those obtained from either nitrogen fixers (*Azospirillum brasiliense*, *Azotobacter chroococcum*, *B. polymyxa*) or *B. circulans* alone). The essential oil percentage and yield per plant for three cuttings was almost two fold higher on fresh weight basis as a result of aqueous extracts of compost at low level + bio-fertilizers compared with control, indicating that combinations of low input system of integrated nutrient management could be beneficial to obtain relatively good yields of essential oil. Essential oil composition using GC/MS revealed that marjoram belongs to the *cis*-sabinene hydrate/terpinene-4-ol chemotype. The chemical composition of marjoram essential oil did not change due to the fertilization type or level; rather the relative percentages of certain constituents were affected. The highest level of *cis*-sabinene hydrate (18.47%) and terpinene-4-ol (24.24%) was obtained with aqueous extracts of compost at 30% + *B. circulans* and aqueous extracts of compost at 30% + (*A. brasiliense* + *A. chroococcum* + *B. polymyxa*), respectively. Most studies of phosphate solubilizing microorganisms involve inoculating the soils (Mishustin *et al.*, 1972). Inoculation with phosphate-dissolvers is claimed to increase the yield of many agriculture crops (Taha *et al.*, 1969; Ewada 1976; Ocampo *et al.*, 1978; Guar *et al.*, 1980; Abdel-Nasser *et al.*, 1982; Subba Rao 1984). Gomaa (1989) demonstrated that, some effects of phosphate solubilizing microorganism's inoculation have been observed in terms of increasing the amounts of available P and plant growth of crop production. Hauka *et al.*, (1990) stated that,

phosphate solubilizing microorganisms significantly increased dry yields and protein content of Barley and Tomato plant. El- Gamal (1996) revealed that, P level or inoculation with phosphorene (phosphate solubilizing microorganisms) significantly increased soil available P, tuber N and P contents and their uptake, foliage dry weight, foliage P content and P uptake, total P uptake and dry matter yield of Potato plants. Applying phosphorene improved growth and P uptake by the Olive seedlings in comparison to the phosphate fertilizer alone (Faisal and El-Dawwy 1999). Applying phosphate solubilizing microorganisms with calcium superphosphate to mustard (*Sinapis alba* L.) plants improved growth, seed yield, lipids content, N, P and their uptake, and protein content but total carbohydrates, soluble sugars and insoluble sugars were decreased, also saturated and unsaturated fatty acids content were changed compared with applying calcium superphosphate fertilizer alone. It recommended that using phosphate solubilizing microorganisms because it increases the production (quantity and quality) and medicinal properties. Also it is very cheap and expressed cash money improving the income of farmer, in addition, uses biofertilizer (phosphate solubilizing microorganisms) is safe for human health (Khalid 2004). Awad and Khalil (2003) reported that, the biofertilizer (*Thiobacillus thiooxidans*) and sulphur significantly increased the growth of squash and raised their nutrient content than Sulphur fertilizer alone. Treated celery (*Apium graveolens* L cv. dulce) plants with different levels of sulphur and sulphuroxidizing bacteria resulted in a significant increase in growth and yield characters, i.e. plant height, branch number, leaf number, umbel number, fresh weight, dry weight and fruit yield/plant in comparison with control plants. Khalid (2005) states that chemical composition analysis of treated plants showed an increase in the essential and fixed oil content, total carbohydrates, crude protein and nutrients content (NPKS) and its uptake. Also treated plants showed an increase in the main components of the essential oil (limonene and β -selinene) extracted from the fruits, comparison to untreated plants.

Evaluate the effect of natural products as a source of some important elements such as rock phosphate as a source of phosphorous and feldspar mica as a source of potassium with biological potassium phosphorous fertilizers or biological potassium phosphorous fertilizers (Silicate bacterium) at different levels (0.0, 25, 50 and 100 g/L) on *Ruta graveolens* L. plant instead of the chemical fertilizers were investigated by Khalid *et al.*, (2007). Adding biological fertilizer with feldspar or rock phosphate improved vegetative growth characters such as plant height (cm), branches number/plant, fresh and dry weights of different plant parts i.e. leaves, stems and roots (g/plant), in addition to some chemical constituents as essential oil, total flavonoides, P, K, Fe, Zn and Cu content. On the other hand, the main constituents of essential oil and N content were decreased compared with adding recommended chemical fertilizers. According to Banchio *et al.*, (2008) the effects of root colonization by plant growth promoting rhizobacteria (PGPR) on biomass, and qualitative and quantitative composition of essential oils were determined in the aromatic crop *Origanum majorana* L. (sweet marjoram). PGPR strains evaluated were *Pseudomonas fluorescens*, *Bacillus subtilis*, *Sinorhizobium meliloti*, and *Bradyrhizobium* sp. Only *P. fluorescens* and *Bradyrhizobium* sp. showed significant increases in shoot length, shoot weight, number of leaf, number of node, and root dry weight, in comparison to control plants or plants treated with other PGPR. Essential oil yield was also significantly increased relative to non-inoculated plants, without alteration of oil composition. *P. fluorescens* has clear commercial potential for economic cultivation of *O. majorana*. In studies on *Coriandrum sativum*, *Anethum graveolens* and *Foeniculum vulgare*, it was shown that AMF root colonization enhances the essential oil quality by altering essential oil components (Kapoor *et al.*, 2002 and 2004). Four organic amendments: leaf compost (LC), vegetable compost (VC), poultry manure (PM) and sewage sludge (SSL) applied at four doses (40, 80, 100 and 120 t ha⁻¹) were evaluated for their effect on the herbage yield, essential oil content and inoculum

potential (IP) of native arbuscular mycorrhizal fungi (AMF) on three varieties of *Java citronella*, *Cymbopogon winterianus* Jowitt (Manjusha, Mandakini, and Bio-13). PM applied at 100 t ha⁻¹ followed by SSL increased the herbage, essential oil content and dry matter yield significantly. Bio-13 performed better and produced the highest herbage, essential oil and dry matter yield. The type and dose of the various organic amendments also significantly influenced the indigenous AMF infectious propagules in soil. Highest number of AMF propagules were recorded in the LC amended plots in all the three varieties. Amongst the varieties, highest native mycorrhizal inoculum was recorded in the Bio-13. Least number of AM infectious propagules was recorded in the Mandakini plants grown in 40 t ha⁻¹ SSL (Tanu 2004). Khaosaad *et al.*, (2006) observed that essential oil levels in *Origanum* species are increased in the presence of arbuscular mycorrhizal fungi. A field experiment was conducted to study and compare the effectiveness of two arbuscular mycorrhizal fungi (AMF), *Glomus macrocarpum* (GM) and *Glomus fasciculatum* (GF) on three accessions of *Artemisia annua*. The AM inoculation significantly increased the production of herbage, dry weight of shoot, nutrient status (P, Zn and Fe) of shoot, concentration of essential oil and artemisinin in leaves as compared to non-inoculated plants. The extent of growth, nutrient concentration and production of secondary plant metabolites varied with the fungus-plant accession combination. The mycorrhizal dependency of the three accessions was related to the shoot: root ratio. Comparing the two fungal inoculants in regard to increase in essential oil concentration in shoot, the effectiveness of GF was more than that of GM. While in two accessions, GM was more effective in enhancing artemisinin concentration than GF. Increase in concentration of essential oil was found to be positively correlated to P-status of the plant conversely (Chaudhary *et al.*, 2008). Five strains of bacteria (1. *Azotobacter chroococcum*, 2. *Azospirillum lipoferum*, 3. *Bacillus polymyxa*, 4. *Bacillus megatherium* and 5.

Pseudomonas fluorescens) were mixed in equal parts and used as biofertilizer in this experiment. The biofertilizer treatment was applied alone or in combination with 1/3, 2/3 or full recommended dose of mineral nitrogen fertilizer. The results indicated that applying biofertilizer treatment alone or in combination with chemical N fertilizer increased the growth, yield and chemical constituents of dill plant compared to the untreated control. The highest values of vegetative growth, oil yield, chlorophyll content and NPK percentages were recorded by the treatment of bio-fertilizer plus two third of recommended dose of nitrogen fertilizer. The lowest values in this respect were obtained by control plants during two seasons. The GC analysis of volatile oil indicated that the main components were carvone, limonene and apiol. These components were affected by biofertilization and chemical N treatments. Partial substitution of mineral nitrogen fertilizer by bio-fertilizer was recommended to increase the yield as well as the quality of dill plant (Hellal *et al.*, 2011). Bio-fertilizer treatments increased the growth characters and essential oil composition of coriander compared with the chemical fertilizers treatments (Hassan *et al.*, 2012). Biofertilizer treatments (mycorrhizal and phosphate bacteria) increased the seed yield and essential oil of fennel plants compared with vermicompost treatments (Darzi 2012). Application of phosphate bio-fertilizer and phosphorus were significant on the vegetative growth characters of *Tagetes erecta* L. plants (Hashemabad *et al.*, 2012). Adding dry yeast at the rate of 6 g/L. was the most effective on growth parameters and oil-percent of *Borago officinalis* plant (Ezz El-Din and Hendawy 2010). An Iranian investigation revealed that inoculation of *Ocimum basilicum* roots with plant growth-promoting rhizobacteria (PGPR) improved growth and accumulation of essential oils. Treatments were *Pseudomonas putida* strain 41, *Azotobacter chroococcum* strain-5 and *Azospirillum lipoferum* strain. In comparison to the control treatment, all factors were increased by PGPR treatments. The maximum Root fresh weight (3.96 g/plant), N content (4.72%) and essential oil yield (0.82%) were

observed in the *Pseudomonas* + *Azotobacter* + *Azospirillum* treatment. All factors were higher in the *Pseudomonas* + *Azotobacter* + *Azospirillum* and *Azotobacter* + *Azospirillum* treatments (Ordoorkhani *et al.*, 2011).

Conclusion

What is the key behind specificity of certain Nitrogen fixing microorganisms to selected plants? Why not other Non-Nitrogen fixing microorganisms acquire the property of nitrogen fixation? Can we evolve nitrogen fixing plants? The search for new microorganisms capable of fixing nitrogen. Exploiting other plant microorganisms associations. Proper utilization of fertilizer nitrogen by means of slow release nitrogen fertilizer. Domestication and cultivation of promising nodulated legume species. Recycling of wastes for elements; microorganisms abundant in the soil and are critical to decomposing organic residues and recycling soil nutrients. The above questions and statements are an outlook of future research.

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