Effects of Vesicular-arbuscular mycorrhiza inoculation on grown *Secale montanum* and *Vicia faba* plants in relation to polluted soils

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ABSTRACT

In the recent years use of sewage sludge as a fertilizer has become prevalent in agricultural lands, because of it is cheap. Sewage sludge is source of nutritional elements.The aims of present study were to The effect of sewage sludge on soil chemical properties and growth of *Secale montanum* and *Vicia faba* with and without present of Arbuscular mycorrhiza fungi. The experiment was conducted in controlled conditions was completely randomized design and factorile test. Three levels of sewage sludge (0, 100 and 200 ton/ha) , two levels of mycorrhiza (with and without mycorrhiza) and two plant (*Secale montanum* and *Vicia faba*) in three replicated were used. Organic matter, total N and P content were measured. The result showed that as the added sewage sludge increased , organic matter, total N and P content in soil with mycorrhizal plants and control ones, but organic matter, total N and P were much higher in soils with mycorrhizal plants. As the added sewage sludge in soil that treated by 100 ton/ha, organic matter, total N and P content in soil and plants tissues increased but organic matter, total N and P in soil and plants tissues were higher in mycoorhizal plants than nonmycorrhizal ones.With increase sewage sludge in soil in treated by 200 ton/ha, organic matter content in treated soil and plants tissue Secale montanum and Vicia faba are increase. Contents of organic matter, total N and P were higher colonized plants than control ones.

INTRODUCTION

Arbuscular mycorrhizal fungi are a natural constituent of the soil of most ecosystems. They interact with the root of more than 80% of terrestrial plants and can be considered functional extensions of plant roots considerably enlarging the volum for nutrient up take(Harrison 1999). Arbuscular mycorrhizal (AM) fungi provide an attractive system to advance plant-based environmental clean up. The arbuscular mycorrhizal fungi (AMF) are universal and ubiquitous rhizosphere microflora forming symbiosis with plant roots and acting as biofertilizers, bioprotactants, and biodegraders During symbiotic interaction the hyphal network functionally extends the root systems of their hosts. AM fungi occur in the soil of most ecosystems including polluted soils by acquiring phosphate, micronutrients and water and delivering a proportion to their hosts they enhance the nutritiona state of their hosts. We know very little about the enormous diversity of soil microbes, their properties, and behaviour in the soil environment. Soil microorganisms inhabiting therhizosphere environment interact with plant roots and mediate nutrient availability, e.g. those forming use-ful symbiotic associations with the roots and con-tribute to plant nutrition. Implications of plants and their symbionts like mycorrhizal fungi, N-fixing rhizobia, and free living rhizosphere population ofbacteria which promote plant growth need to be fully exploited and encouraged by inoculating nutrient poor agricultural soils with appropriate microbes (Khan, 2002a).

MATERIAL AND METHOD

The experiment was conducted in controlled conditions was completely randomized design and factorile test. Three levels of sewage sludge (0, 100 and 200 ton/ha) , two levels of mycorrhiza (with and without mycorrhiza) and two plant (Secale montanum and Vicia faba) in three replicated were used. These AM fungal species were identified morphologically using current taxonomic criteria and Internet informatttion by INVAM (http://invam.caf.wvu.edu/). Propagated on sudangrass *Sorghum sudanese*. Grown in a sandy soil for three successive propagation cycles each 4 mo long.The inocula were airdried and sieved (2 mm), and each consist of a mixture of rhizospheric soil from a pure pot culture containing spores, hyphae, and mycorrhizal root fragments. The data were analyzed using statistical software (SPSS). This experiment was in october 2010-2011 in greenhouse of Faculty of Agriculture,Shahid Chamran University Ahvaz, located in Iran, due to study of the Heavy metal uptake by arbuscular mycorrhizas of *secale montnum* and *vica faba* grown in contaminated soil with sewage sludge.

Soil Preparation

Soil sample collected from the farm of Faculty of Agriculture, Shahid Chamran University in Iran. One sample of the soil (depth 10-15 cm) was taken and sieved through a 2-mm sieve large stone and plant root debris were removed. The soil was then thoroughly mixed for the following experiment Then Sample of dried sewage sludge sieved through a 2 mm sieve. The physical and chemical characterizes of soil and sewage sludge estimate. The Soil texture is loamy silt, with the following properties: Soil pH (soil: water ratio, 1:2.5) 7.75, 1.3% organic matter, 0.0725% total N, 66 mg kg^{-1} Olsen P, 340.5mg kg^{-1} M NH_4OAC extractable k, 0.46 mg kg^{-1} total Cu, 0.73 mg kg^{-1} total Mn, mg kg^{-1} total Pb, 0.072 mg kg^{-1} total Cd, 0.199 mg kg^{-1} total Ni. The soil extractable HMs were extracted by 0.1 M HCl(Lu, 2000), and the concentrations HM in sewage sludge were as follows: , 14.76 mg kg^{-1} Cu, 69.72 mg kg^{-1} Mn, 2.162 mg kg^{-1} Pb, , 0.638 mg kg^{-1} Cd. , 0.199 mg kg^{-1} Ni Sewage sludge pH 6.8, 11.5% organic matter, 1.8% total N, 286 mg kg^{-1} total P.

Pot Experiment

The pot experiment consist of Three levels of sewage sludge (0, 100 and 200 ton/ha) , two levels of mycorrhiza (with and without mycorrhiza) and two plant (*Secale montanum* and *Vicia faba*) in three replicated. Fifty gram of inoculums was mixed uniformly with soil and filled into each pot. The seed of *Secale montanum* and *Vicia faba* were surfacesterilized with 0.5% NaClo solution and subsequently washed several times distilled water and only the seed of Vicia faba germinated at 28 ^{0}C(for 48 h) before sowing. Pot were arranged in completely randomized design and factorile test with three replicated. Plants were grown in a sunlit greenhouse with natural light, a day/night temperature 33 ^{0}C, and a relative humidity 40%-60%. Plants were watered to maintain soil moisture at 70% of the field capacity (FC). Shoot and root were harvested separately 10 week after sowing. Subsample s of fresh roots were taken to assess mycorrhizal colonization. The fresh weight of total roots and subsamleds were measured. Shoots and remaining roots were first rinsed with tap water and then with deionized water, theb weighted after oven drying at 70 ^{0}C 40 for 48 h. Mycorrhizal colonization were evaluated by the grid-line intersect method (Giovannetti and Mosse, 1980). After cleaning with 10% KOH and staining with acid fuchsin (Phillips and Hayman, 1970). Ni and Cd concentrations in dried and ground plant material were determined by atomic after wet digestion with a mixture of concentration HNO_3 and $HCLO_4$. HM concentration of roots and shoots were calculated.

Statistical analysis

The data were subjected to one-way ANOVA using the SPSS 16.00 software. Means and standard errors were calculated. Means were compared by the Duncans multiple range test*($P<0.05$)* and *$P<0.01$).*

RESULTS AND DISCUSSION

The total N content of soils treated with sewage sludge

The result showed that as the added sewage sludge is increased , total N content both mycorrhizal plants and control ones, but total N was higher in non-mycorrhizal plants (the difference is non significant). As the added sewage sludge in soil that treated by 100 ton/ha, total N content in soil increased but total N in soil pkanted with *Secale montanum* and *Vicia faba* were higher in soils with non-mycoorhizal plants than mycorrhizal ones.With increase sewage sludge in soil in treated by 200 ton/ha, total N content in treated soil with *Secale montanum* and *Vicia faba* are increase. Contents of total N were higher non mycorrhizal plants than mycorrhizal ones (figure 1, 2).

Table 1 There were significant and non significant effect sewage sludge on total N content of soil, planted with inoculated (M) *Vicia faba* and *Secale montanum* related to the non inoculated (NM).

Treatment	df	SS	MS	P valu
plant	1	0.017	0.017	**35.895
mycorrhiza	1	0.007	0.007	**15.239
Sewage sludge	2	0.555	.0278	**579.968
mycorrhiza × plant	1	0	0	ns0.697
sewage× plant × mycorrhiza	2	0.007	0.003	**6.976
sewage ×plant ×mycorrhiza	2	0.003	0.002	ns3.141
sewage	2	0.001	0	ns0.788
Error	24	0.011	0	

p<0/01** , p<0/05* & ns (non significant)

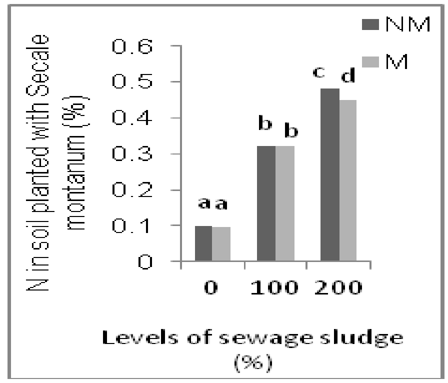

Fig 1 Change in contents of total N in soil planted with inoculated (M) and non inoculated (NM) *Secale montanum*

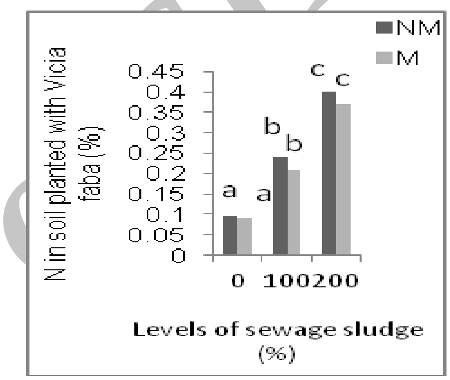

Fig 2 Change in contents of total N in soil planted with inoculated (M) and non inoculated (NM) *vicia faba*

KEYWORDS: Arbuscular mycorrhizal, soil chemical properties

CONCLUSIONS

Adding sewage sludge to soil increased consumption of high-nitrogen plant. The primary form of inorganic nitrogen in the sludge NH^{4+} that almost 30 percent of total nitrogen in the anaerobic sludge. The addition of sludge to soil type, soil total nitrogen will increase sewage sludge can be a big part of the nitrogen required to provide products. Nitrogen from sewage sludge is added to the soil should be process plant to be absorbed. Sewage sludge contains significant amounts of organic and inorganic nitrogen. After adding the soil can to enhance soil nitrogen . And organic nitrogen mineralization process also gradually taken up and converted to nitrate nitrogen . Go et al (1992) reported that the addition of sewage sludge to soil, increase soil P. One of the effects of sewage sludge, soil organic matter is increased. So that the sewage sludge can increased the organic matter . Many other effects of sewage sludge on soil properties can be caused by the organic matter of sewage sludge.The extra-radical mycelium of AMF, in addition to its crucial role in enhancing nutrition of host plant, also plays a role in soil particle aggregation, soil fertility and soil stability (Dodd et al., 2000; Wright and Upadhyaya,1998). AM associations are important in natural andmanaged ecosystems due to their nutritional and non-nutritional benefits to their symbiotic partners. They can alter plant productivity, because AMF can act as biofertilizers, bioprotectants, or biodegraders (Xavier and Boyetchko, 2002). AMF are known to improve plant growth and health by improving mineral nutrition, or increasing resistance or tolerance to biotic and abiotic stresses (Clark and Zeto, 2000; Turnau and Haselwandter, 2002). AMF improve P-nutrition by scavenging available P through the large surface area of their hyphae. PGPR (plant growth promoting rhizobacteria) may also improve plant Pacquisition by solubilizing organic and inorganic P sources through phosphatase synthesis or by lowering pH of the soil (Rodríguez and Fraga, 1999).

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