# STUDY OF BIODIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN ADDITION WITH DIFFERENT ORGANIC MATTER IN DIFFERENT SEASONS OF KAVRE DISTRICT (CENTRAL NEPAL)

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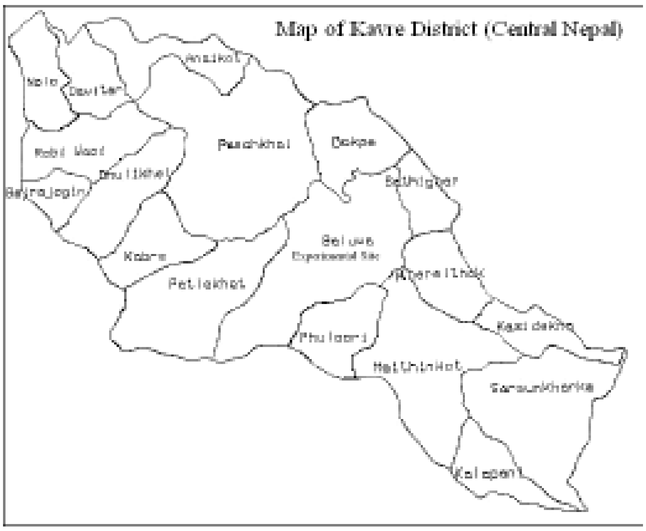
**Abstract:** Different types of organic matter (dried and powdered 5 gms. leaves of *Tithonia diversifolia, Eupatorium adenophorum, Lantana camara*, farmers compost) and triple-superphosphate were mixed with eroded soil collected from sites with no vegetation. Eroded soil without amendments served as control. The mixture was placed in mesh bags (50 Mm mesh) that allowed fungal colonization but excluded roots. Two sets of mesh bags were buried around *Bauhinia purpurea and Leucaena diversifolia* that had been planted in an eroded site. The first set was buried in June 2003 and harvest in December 2003 (the monsoon period) and the second set was buried in December 2003 and harvested in June 2004 (The drier period). We found that more Arbuscular mycorrhizal (AM) fungal biomas and spores were produced during the wet season than during the dry season. The different types of organic matter had similar influence on the amount of AM biomass but the species composition was varied with the types of organic matter. In wet season nine species of AM spores and in dry season ten species of AM spores were found. In dry season *Scutellospora nigra* was found which was different from wet season.

**Key words:** Arbuscular mycorrhiza; Organic matter; Wet season; Dry season.

## INTRODUCTION

Nepal is very prone to soil erosion and is susceptible to sediment disasters mainly caused by slope failure, land slides, debris and bank erosion. There is an urgent need to control erosion and prevent potential sediment disaster in Nepal (Shrestha Vaidya et al. 2002). Therefore mycorrhizae are essential for the establishment of tree seedlings and for their good growth and development in soils in low nutrients.




Because mycorrhizae is beneficial to tree growth as they increase nutrient uptake and expose a greater absorbingsurface (Shrestha Vaidya et al. 2002 and 2005).

The arbuscular mycorrhizal (AM) symbiosis is an association between most terrestrial plants and a class of fungi (Glomeromycota) which occurs in the roots of host plants (Schussier et al. 2001). AM fungi are normally considered to improve plant mineral nutrition( in particular phosphorus (P), water uptake, and resistance to root pathogens (Smith & Read 1997).

Addition of organic matter such as green manure is a common practice to improve soil nutrient content and soil structure . Organic residues from plants such as *Tithonia diversifolia* and *Lantana camara* have been found to be especially beneficial since they are reported to have a high content of N and P, which is mineralized rapidly from the organic material. Nziguheba et al. (2000) found that P is released more rapidly from such organic residues than from triple superphosphate.

Successful colonization by mycorrhizal fungi is especially important in degraded soils where nutrient availability is low; furthermore, AM fungi improve soil structure because they produce extraradical hyphal networks and their hyphae contain and release glomalin, which is a putative glycoprotein, assayed from soil as glomalin-related soil

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protein (GRSP) that is correlated with aggregate water stability (Wright & Upadhyaya 1998, Rillig 2004; Rillig & Mummey 2006). Improved soil structure increases water infiltration and can reduce soil erosion (Tisdall & Oades 1982).

Many biotic and abiotic factors influence growth and biomass partitioning of AM fungi. Farming systems (Boddington & Dodd, 2000), soil moisture (Anderson et al. 1983), organic matter (Ryan et al. 1994), pH (Porter et al. 1987; van Aarle et al. 2002) and temperature (Koske 1987) are all examples of factors that can influence the distribution of AM fungal hyphae and spores. Managing soil could thus be a potential way to optimise proliferation of indigenous AM fungi (Boddington & Dodd 2000). Particularly, addition of organic matter can have a beneficial effect on the growth of indigenous AM fungi in nutrient-limited soils (Caravaca et al. 2002; Gaur & Adholeya 2002). Organic amendments enhance spore production (Johnson & McGraw 1988; Douds et al. 1997), extra radical proliferation of hyphae (St. John et al. 1983; Joner & Jakobsen, 1995), and improve colonization of roots (Muthukumar & Udaiyan 2000). Giovanetti & Avio (1985) suggested that this beneficial effect might be related to increased pore volume in soil which has a beneficial effect on AM colonization, the mycorrhizal growth response and AM spore numbers. Furthermore, Ryan et al. (1994) attribute increased AM fungal biomass to the beneficial effects of organic matter on soil structure, water status, and on synergistic microbial activities in the soil. Organic matter addition to the soil in eroded sites could thus be an approach to enhance the beneficial effect of AM fungi on soil stabilization and plant establishment.

## MATERIAL AND METHODS

This field experiment was conducted in Baluwa Forest Kavre district in Central Nepal.

This forest is situated 40 km East of Kathmandu city on the side of Arniko highway to the Tibetan border. The study site was completely eroded as a result of a land slide in 1998 it was planted with *Bauhinia purpurea* and *Leucaenia diversifolia* which both are important fodder plants in Nepal. The Fodder department of the National Agriculture Research Council, Khumaltar, Nepal, performed Plantation. At the time of our experiment approximately 30 % of the plants had survived. The soil at the experimental site is in this area are dominated by Rhodustults and Hapolustults (both members of the Ultisols soil order) (Brown et al. 2000). The chemical characteristics of the soil have been examined earlier by the Division of Soil Science, Nepal Agricultural Research Council (NARC). It was found that the soil was acidic (pH 4.0) and poor in nutrients and in organic matter. The total content of N, measured by the Kjelldahl method, was 4.1 mg $g^{-1}$ and the organic matter content was 0.73%. The low pH and the high content of Al and Fe in these soils suggest a very high P fixing capacity G.Shrestha Vaidya et.al 2007).

### *Experimental design and methods*

Growth of AM fungi under field conditions was estimated with in-growth mesh bags, similar to a design used earlier to estimate growth of mycorrhizal fungi in forests (Wallander et al. 2001), sand dunes (Olsson & Wilhelmsson 2000), (G.Shrestha Vaidya et.al 2007) and pre-Saharan desert shrubland ecosystems (Labidi et al. 200x). The mesh bags were constructed of nylon mesh (50 µm mesh size) to allow fungal colonization but excluded roots, because the latter cannot penetrate the mesh. These bags were used to clearly separate hyphal from root effects in the field. The mesh bags were filled with eroded soil mixed with different forms of organic matter or rock phosphate (see below).

The eroded soil was collected from a degraded site at Bisankhu Narayan (Godavari) in Nepal. This site was completely eroded due to a landslide after heavy rainfall and no vegetation was present on the site. Forty-five g of eroded soil were placed in nylon mesh bags and mixed with different types of organic matter or left unmixed as control: 5 gms. of dried leaves or dried compost (10% by weight) was used as organic matter additions. Fully expanded leaves of three common agroforestry plant species (*Tithonia diversifolia, Lantana camara* and *Eupatorium adenophorum*) were collected from border rows in a farmer's fields and from roadsides and the compost were collected from a local farmer. The compost was made from disposed vegetable waste, cow dung, straw and husk. One of the treatment Triple-superphosphates. In this case 45 mg Trple-superphosphate was mixed with 50 g of eroded soil. This represents approximately 40 – 50 kg P per hectare which is an amount usually used by local farmers. The mesh bags that were used as controls were filled with 50 gms. of eroded soil without any amendments. In total, 6 treatments were included (control, *Tithonia diversifolia*, *Lantana camera*, *Eupatorium adenophorum*, compost and Triple-superphosphate).

The experiment lasted for one year: one set of mesh bags was buried from June 2003 through December 2003 (the monsoon period) and a second set was buried in the same locations from December 2003 through June 2004 (the dry period). During the dry period some rain was recorded in January (2-3 days) and some rain was recorded in June (2-3 days). Plenty of rain was recorded during the wet season although no estimates of the amounts were made. Mesh bags containing each of the six treatments were buried 10 cm from the base of eight *Bauhinia purpurea* trees and eight *Leucaena diversifolia* trees (approximately 1.5-2m high) for a total of eight replicates in each tree species. A total of 192 bags were used (2 harvests x 6 treatments x 2 tree species x 8 replicates). Spore production differed between tree species and the tree species are therefore separated in the spore analysis. The mesh bags were buried to a depth of about 10 cm where the density of roots was high (Shrestha Vaidya et.al. 2007).

### *Elemental analysis of Plant and Soil material*

The fresh leaves of the plant species were air dried and ground to pass a 0.5 mm sieve. The Concentrations of Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn of dried plant leaves, dried compost and eroded soil were analyzed with ICP-AES. The C and N were analyzed with an elemental analyzer (Elementar Analysensysteme GmbH. Modell vario MAX CN.).

**Table 1:** Chemical composition mg $g^{-1}$of the eroded soil and the different organic amendments used in the mesh bags. Values are one measurement of a pooled and well-mixed sample of each substrate.

| Sample type | C | N | P | C:N | C:P | K | Ca | Mg | S | Al | Fe |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Thitonia diversifolia* | 441 | 33.2 | 2,8 | 13.2 | 158 | 34.1 | 13.4 | 3.1 | 1.8 | 0.2 | 0.2 |
| *Lantana camara* | 414 | 28.6 | 1.7 | 14.5 | 243 | 12.2 | 26.3 | 2.9 | 2.6 | 0.7 | 0.6 |
| *Eupatorium adenephorum* | 464 | 36.7 | 2.6 | 12.6 | 178 | 22.6 | 14.8 | 2.2 | 2.0 | 0.5 | 0.6 |
| Farmers Compost | 247 | 22.3 | 17.6 | 11.0 | 14.0 | 14.7 | 15.5 | 11.8 | 4.1 | 2.2 | 2.8 |
| Eroded Soil | 1.4 | 0.11 | 0.3 | 12.4 | 0.4 | 1.0 | 1.1 | 0.6 | 0.03 | 12.5 | 42.3 |

## Spore analyses

The AM fungal spores within 25 g of the soil and amendment mixtures inside the mesh bags were extracted, identified and quantified. Spores were extracted using wet sieving and sucrose centrifugation (McKenney & Lindsey 1987). Spores were mounted in polyvinyl alcohol on slides and examined using a compound microscope. Species were identified to species using taxonomic characteristics described on the INVAM website (http://invam.caf.wvu.edu/) and Schenck & Perez (1990). Several spore samples from the first harvest were lost, especially from the plots with *Leucaena diversifolia* trees. In total 43 spore analysis were analysed from the first harvest (wet season) and 96 from the last harvest (dry season) (G.Shrestha Vaidya et.al 2007). The potential to find effects of the different organic material on spore composition is therefore smaller for the first harvest compared to the second harvest.

### Analysis of Endomycorrhizal Fungi (AM spores) in different seasons

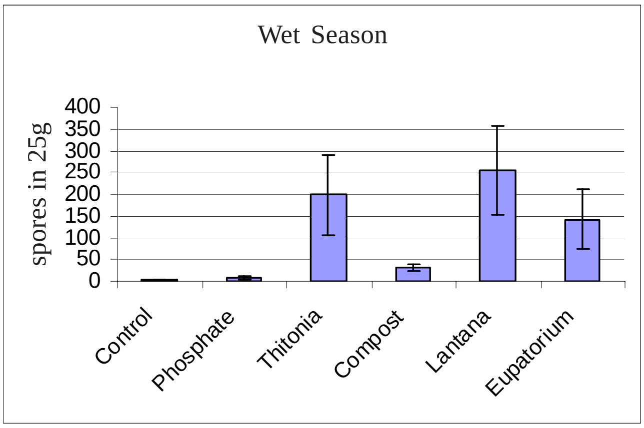



**Fig. 1**

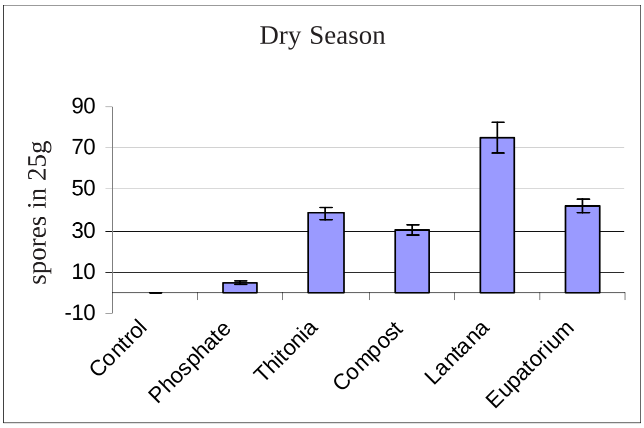
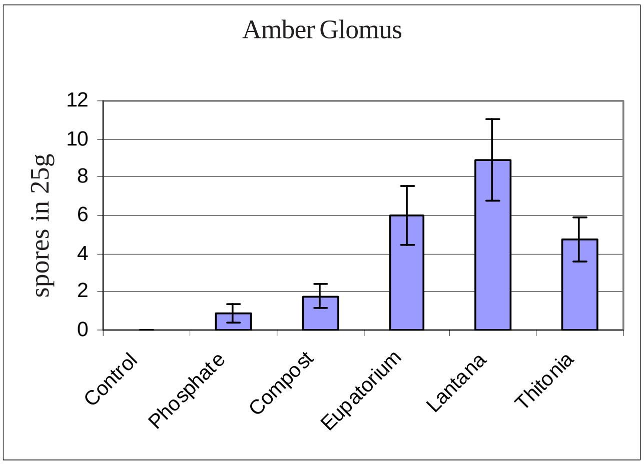



**Fig. 2**

### Analysis of different Endomycorrhizal Fungi (AM spores) in different organic matter

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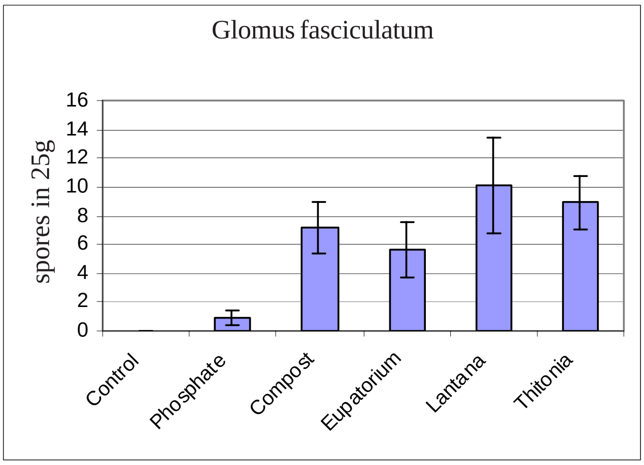


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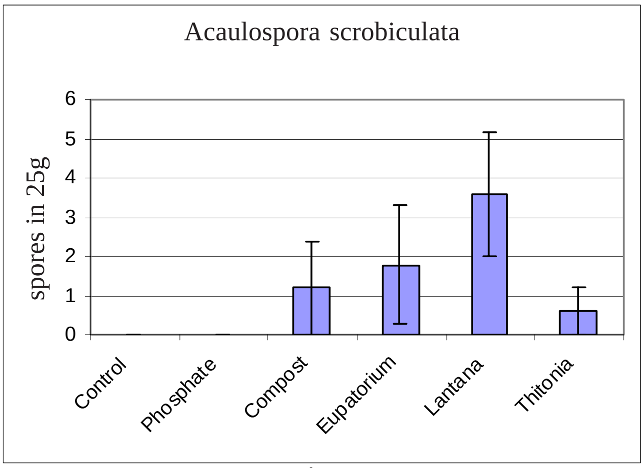


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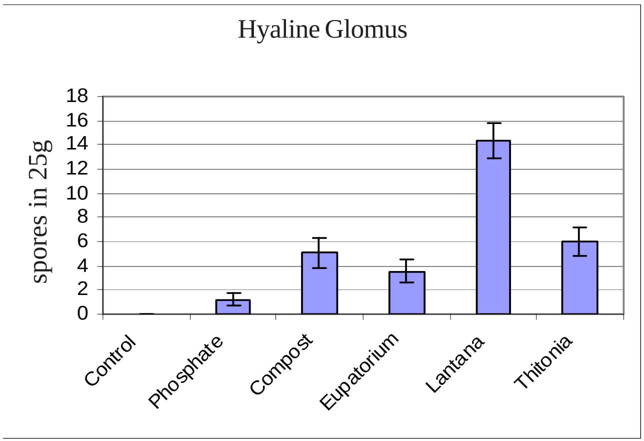


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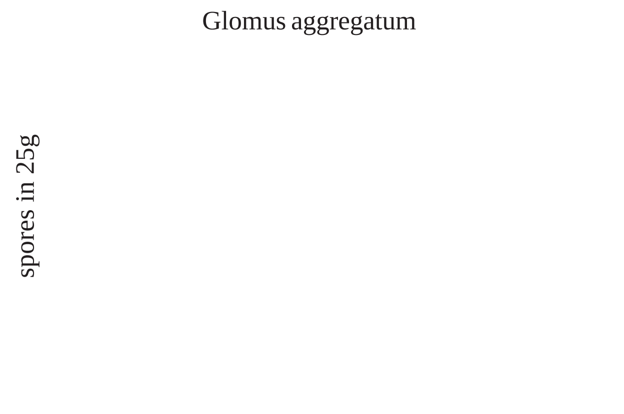


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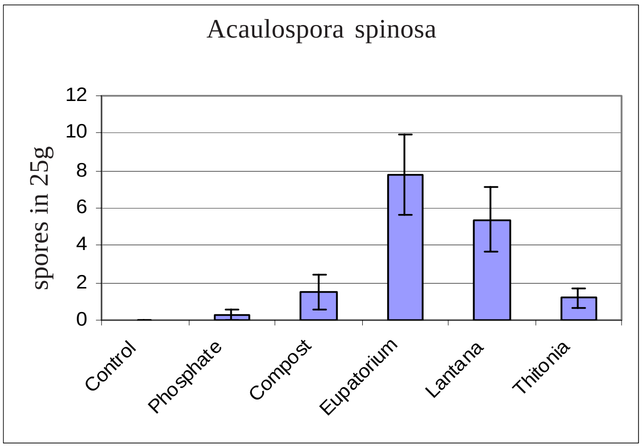


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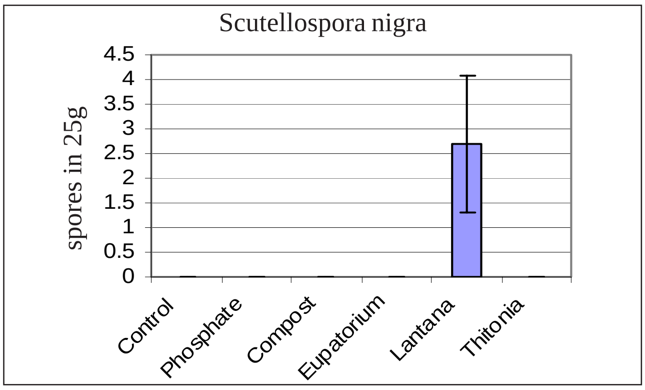


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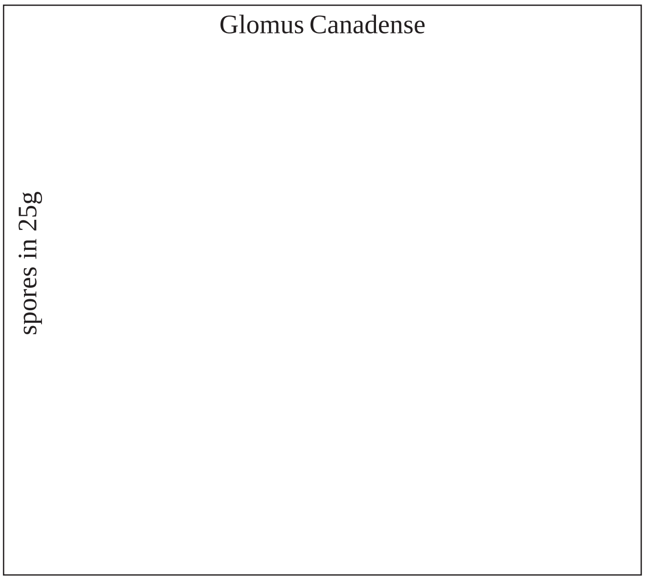


**Fig. 10**

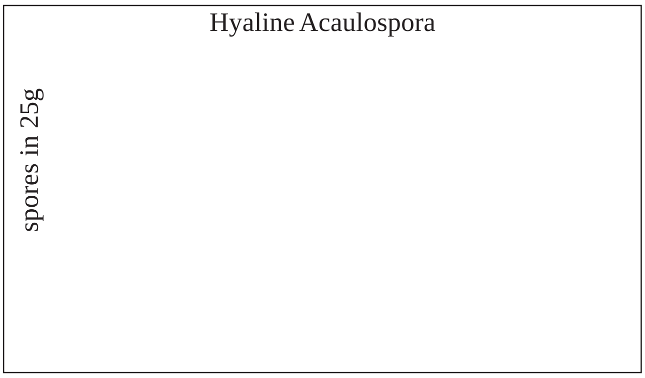


**Fig. 11**

## RESULTS

The eroded soil that was used in the mesh bags had extremely low levels of C, N, P, K, Ca, and Mg and high levels of Fe and Al. The dried leaf material from the three agro forestry species that was used as organic amendments in the mesh bags differed somewhat in chemical content (Table 1). *Eupatorium* and *Tithonia* appeared to be more similar while *Lantana* had lower N and P content. The compost had considerably higher P content than the dried leaf material (Table 1).

The compost contained much more P than the leaves from the agro forestry plants AM fungi produces significantly lower amounts of spores in the mesh bags with compost compared to the other treatments, which may indicate that spore formation was inhibited by the high P level in the compost. We found no effect of triple-superphosphate addition and the effect of triple-superphosphate addition on spore formation cannot be evaluated since spore formation in mesh bags without organic matter addition was almost absent. More spores were found in wet season than dry season but in dry season one species *Scutellospora nigra* was found but not found in wet season. In both seasons number of spores is more in *Lantana camara* than other organic matter (Fig.1 & Fig. 2). The different number of AM spores in different organic matter was found different in number ( Fig. 3 to Fig. 11). The addition of compost or green manure is an important way to improve the soil in degraded areas since nitrogen and other nutrients, as well as organic matter which improves soil structure, is added with the organic material (Caravaca et al. 2002; Muthukumar & Udaiyan, 2000, Nziguheba et al. 2000).

## DISCUSSION

The use of in-growth mesh bags was found to be a successful way of measuring recently produced AM fungal biomass and spores in eroded slopes of Nepal vegetated with *Bauhinia purpurea* and *Leucaena diversifolia*. Other methods for estimating biomass of AM fungi in soil such as extraction of hyphae or spores (Boddington & Dodd 2000) or estimates of biochemical markers directly in soil samples (Olsson et al. 1999) includes an unknown fraction of dead or inactive AM biomass. The production of recently formed extra radical AM mycelia is an important parameter since it can be directly related to the capacity of the plants to take up nutrients and to improvements of the soil structure and stability in degraded soils.

Similar results were found by adding compost made of Acacia cyanophylla leaves to eroded soil in Acacia tortilis savanna in pre-Saharan areas in Tunisia (Labidi et al. 2006). The positive effect of organic matter addition on AM growth could be an effect of higher humidity in mesh bags with organic amendments, since the addition of organic matter has a beneficial effect on soil structure and water-holding capacity (Ryan et al., 1994). The added organic matter could also increase the soil porosity and decrease the mechanical soil resistance to the growth of AM hyphae (Joner & Jakobsen, 1995).

Improved nutrient and water uptake by the planted trees can be expected in response to better AM growth and the positive effect on the growth of AM fungi is in good and found that organic matter addition increased AM fungal hyphal growth (Labidi et al 200x; Nicolson 1959; Koske et al. 1975; Joner & Jakobsen, 1995) and AM spore formation (Douds et al. 1997; Baby & Manibhushanrao 1996; Muthukumar & Udaiyan, 2000; Gryndler et al. 2002; Harinikumar & Bagyaraj 1989; Jamil Mohammed et al. 2003; Jeffries & Barea (2001). In addition, St John et al. (1983), Frey & Ellis (1997) and Friberg (2001) found that AM fungal hyphae grew best in soils with a high amount of organic matter. The highest VAM fungal population was recorded in November and decreased thereafter. The low VAM fungal population in January and February could be due to low soil moisture (Khadge 1988). Same way in our study also we got more spores in wet season than dry season.

The present study provides the first information on a stimulating effect of organic material addition on extra-radical growth of AM fungi in eroded slopes in Nepal (G.Shrestha Vaidya et.al 2007). These results show that organic matter addition can improve AM spores as well as plant survival in such areas.


## ACKNOWLEDGEMENT

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