CHAPTER 4
IMPACT OF AM FUNGAL INOCULATION ON GROWTH AND BIOMASS OF VETIVERIA ZIZANIOIDES (LINN.)

SECTION 1
INTRODUCTION

The success of mycorrhizal evolution has been attributed to the role that mycorrhizal fungi play in the capture of nutrients from the soil of all ecosystems (Bonfante and Perotto, 2000). Literally, “mycorrhiza” means fungus root and is derived from the Greek word “Mykes” meaning fungus and “Rhizo” meaning root (Friberg, 2001). This term was first used by Frank, a German Plant Pathologist in 1855 to describe the symbiotic relationship between plant roots and fungi. The symbiosis is characterized by the exchange of nutrients where carbon in the form of hexose sugars flows to the fungus and inorganic nutrients are passed to the plant, thereby providing a linkage between the plant root and the soil (Sylvia et al., 1998). Mycorrhizal fungi provide inorganic nutrients mainly phosphorus and other complexed compounds to the plant through the extensive network of their hyphae that forage for soil nutrients more effectively than plant roots (Van der Heijden et al., 1998b). For this association to occur there must be a host plant (the phytobiont), an ecological habitat (the soil) and a suitable fungus (the mycobiont). Mycorrhizal fungi differ from other plant–fungus
associations because of their ability to create an interface for nutrient exchange which occurs within living cells of the plant (Brundrett, 2004; Brundrett, 2002). Over 80% of plant species are associated with mycorrhizal fungi, amongst which are vascular and non-vascular plants and some important crops such as carrots, maize, leek, coffee, cocoa, soybeans, apples, citrus fruits, tomatoes and pepper to mention a few (Muchovej, 2004; Bonfante and Perotto, 2000). Mycorrhizal fungi interact with plants at different levels and can be grouped into obligately mycorrhizal, facultatively mycorrhizal and non-mycorrhizal plants (Brundrett, 2004). Facultative mycorrhizal plants as the name denotes, are not solely dependent on the fungus for phosphorus or other nutrients, but can also derive their nutrients from the soil when soil phosphorus levels are high. Thus, this level of association is dependent on soil fertility as mycorrhizal plants can reduce their association with the fungus in cases where the association provides little benefit (Brundrett, 2004; Koide and Schreiner, 1992). Obligate mycorrhizal plants are solely dependent on mycorrhizal fungi for their phosphorus nutrition, as such both plant, and the fungus associate closely with each other. Some non-mycorrhizal plants belonging to the families Amaranthaceae, Brassicaceae and Caryophyllaceae are less attractive to mycorrhizal fungi but at times, attempts are still made to colonize their roots (Brundrett, 2002, Ocampo et al., 1980). The inability of these plants to support mycorrhizal colonisation may be due to the accumulation of chemicals like alkaloids, cyanogenic glucosinolates and antifungal compounds in the roots which fail to elicit differential hyphal branching (Brundrett, 2002; Giovannetti and Sbrana, 1998). Some of these plants like mangel and canola function independently in terms of nutrient acquisition.
through the use of their root system to modify the pH of the rhizosphere and increasing nutrient availability in the soil (Brundrett, 2002; Brundrett, 2004).

There are different types of mycorrhizal interactions which have been classified into ectomycorrhiza and endomycorrhiza based on the presence of various extraradical or intraradical hyphal structures (Bonfante and Perotto, 2000). Seven mycorrhizal types have been identified but the most common endomycorrhizas are the arbuscular mycorrhizas (Brundrett et al., 1996). Other types such as Ectendomycorrhiza, Arbutoid and Monotropoid mycorrhizas are grouped under ectomycorrhizas. These are characterised by the formation of a hartig net and a mantle or sheath around the plant roots. Orchid and Ericoid mycorrhizas are other forms of endomycorrhizas that are known for their ability to penetrate the outer root cells to form intracellular hyphal coils, swellings, or branching. They differ from the arbuscular mycorrhizal fungi by having septate hyphae that are restricted to the epidermal cells of plant roots (Molina et al., 1992). All these mycorrhizal types differ from each other by the characteristic host plant that they associate with, fungal species involved and morphology within roots (Prescott et al., 2005; Brundrett, 2002).

Recent research on arbuscular mycorrhizas has demonstrated that AM fungi play a significant role in plant phosphorus (P) uptake, regardless of whether the plant responds positively to colonization in terms of growth or P content. In this chapter we focus particularly on implications of this finding for consideration of the balance between organic carbon (C) use by the fungi and P delivery (i.e. the C–P trade between the symbionts). Positive growth
responses to arbuscular mycorrhizal (AM) colonization are attributed frequently to increased P uptake via the fungus, which results in relief of P deficiency and increased growth. Zero AM responses, compared with nonmycorrhizal (NM) plants, have conventionally been attributed to failure of the fungi to deliver P to the plants. Negative responses, combined with excessive C use, have been attributed to this failure. The fungi were viewed as parasites. Demonstration that the AM pathway of P uptake operates in such plants indicates that direct P uptake by the roots is reduced and that the fungi are not parasites but mutualists because they deliver P as well as using C. We suggest that poor plant growth is the result of P deficiency because AM fungi lower the amount of P taken up directly by roots but the AM uptake of P does compensate for the reduction. The implications of interplay between direct root uptake and AM fungal uptake of P also include increased tolerance of AM plants to toxins such as arsenate and increased success when competing with NM plants. Finally we discuss the new information on C–P trade in the context of control of the symbiosis by the fungus or the plant, including new information (from NM plants) on sugar transport and on the role of sucrose in the signaling network involved in responses of plants to P deprivation.
SECTION 2
REVIEW OF LITERATURE

Work done by various workers recently indicates the changing understanding of interactions between fungi and plants in arbuscular mycorrhizal (AM) symbioses, particularly those involving nutrient transfers between the living cells of the symbionts. The most important of these transfers are organic carbon (C) delivery to the fungi by the plants and phosphorus (P) delivery in the opposite direction.

In this discussion two important starting positions are taken. First, that in almost all natural environments (including agricultural ones) the majority of terrestrial plant species (perhaps 90%) form mycorrhizas, with the most common type being arbuscular mycorrhizas2 (Smith and Read 2008). This means that the non mycorrhizal (NM) condition should be viewed as abnormal for the majority of species and that a changed perspective is required to regard plant responses to experimental NM treatments as a consequence of removal or prevention of the symbiosis in normally AM plants. This reverses the current perspective that often regards AM formation as an optional add-on to NM controls. Second, that the conventional emphasis on the role of the plant in controlling the symbiosis and its outcomes in terms of plant growth responses requires re-evaluation.

As obligate symbionts AM fungi clearly obtain essential benefits from the symbiosis and have key and possibly controlling roles in symbiotic
development and function (Fitter 2006, Helgason and Fitter 2009, Smith et al. 2011, Smith and Smith 2011a). Approximately 200 morphospecies of AM fungi have been described, but there is much more genetic and functional diversity in the group than this small number suggests (Bever et al. 2001, Helgason and Fitter 2009). There is also marked diversity among AM fungal communities below-ground, whether associated with different vegetation, soil types or seasons, different plant taxa or a combination of these factors (e.g. Bever et al. 2001, Öpik et al. 2006).

As with their hosts AM fungi are subjected to strong evolutionary forces and have their own interests in growth and survival; these interests often are overlooked when the focus is on the ecology or agronomy of the plants.

**Phosphorus uptake**

The best-known aspect of AM function is the role played by AM fungi in enhancing plant P acquisition from soil. This activity is set against transfer of sugars in the form of recent photosynthate from plant to AM fungus so that the balance of trade of organic C for P is a major influence on the outcome of the symbiosis for the plants. It also may influence fungal success, but almost nothing is known of this possibility. Phosphorus is an essential nutrient for all organisms, including plants and fungi, but is difficult for them to obtain from soil (Schachtman et al. 1998, Smith and Smith 2011a). The dominant form absorbed is orthophosphate (Pi, H2PO4^−), which reacts strongly with calcium in soils of high pH and with iron and aluminium at low pH. Hence available P in soil is normally much lower than
total P and Pi concentrations in the soil solution are normally low (less than 10 mM); this is about 1000-fold lower than cellular concentrations.

Furthermore because Pi is negatively charged and cell membranes have electrical potential differences that are inside-negative there is a steep electrochemical potential gradient against which uptake occurs. Uptake therefore is strongly energy-dependent (active) in both fungi and plants. Plants have an additional problem in that uptake of Pi by roots often occurs at a faster rate than it can be replaced by diffusion from the bulk soil. This imbalance leads to depletion of Pi in the rhizosphere that might further limit Pi uptake. In consequence the rate at which roots grow into undepleted soil plays a major role in determining the effectiveness of P acquisition (Tinker and Nye 2000). It therefore is not surprising that plants have evolved a number of strategies that either increase the effectiveness with which they can scavenge P from the soil (e.g. rapid root growth, root-hairs and arbuscular mycorrhizas) or release P from insoluble sources by various mechanisms, essentially mining soil P reserves (e.g. ecto and ericoid mycorrhizas or cluster roots) (Lambers et al. 2008).

**Arbuscular Mycorrhizas: An Overview**

Arbuscular mycorrhizas, which are symbiotic associations between underground organs (mainly roots) and members of the fungal phylum Glomeromycota (Schüßler et al. 2001), are the most common and widespread of the strategies that increase the Pscavenging ability of plants. The symbiosis is ancient and almost certainly was a key factor in plant colonization of land more than 450 000 000 y ago (Smith and Read 2008).
This type of mycorrhiza has three essential components: the root itself, plus AM fungal mycelia that develop inside and outside the root. These mycelia live in distinct environments. Inside the root the intraradical fungal infection units grow within the root apoplast, both in the intercellular spaces and in specialized compartments formed by invagination of cortical cell plasma membranes. The fungal structures form symbiotic interfaces with the plant across which nutrient transfers occur; these interfaces are composed of the plasma membranes of both symbionts and an apoplastic compartment between them. Outside the root in the soil extensive, branched, external mycelium grows from the infection units (see Smith and Read 2008). A single root axis might contain two or more infection units per centimeter on average (see Harley and Smith 1983, p 51), each producing an external mycelium that can extend many centimeters beyond the rhizosphere and potentially colonize different parts of the same root system and other plants. Lack of tight specificity in the symbiosis with respect to choice of partners means that a single root may be colonized by several AM fungi in nature (e.g. Ö pik et al. 2006). This provides the potential for both complementary and antagonistic activities, given the functional diversity exhibited by different AM fungi in symbiosis with the same and different plant species (e.g. Jakobsen et al. 2002, Jansa et al. 2008, Munkvold et al. 2004 and Smith et al. 2000).

The internal mycelia grow in an apoplastic root compartment (the interfacial apoplast), which is bounded by the membranes of both symbionts and contains varying amounts of plant and fungal wall material (the interfacial matrix). Conditions in the interfacial apoplast probably are relatively
constant, but conditions (such as pH and solute concentrations) may be modified by activities of both symbionts.

Unfortunately little is known of this highly important region of the symbiotic root. In contrast the external mycelium will be subject to enormous variations in conditions, depending on soil type, nutrient status, pH, seasonal variations in temperature etc. However, unlike mycelia of saprotrophic fungi, the external mycelium is not limited in its growth by availability of soil organic C. From a fungal perspective the internal mycelium is the critical phase via which the obligate symbionts obtain organic C from the plant and use it to support growth both within the root and in the soil. The external mycelium also can forage for new sources of C by colonizing other plants (Olsson et al. 2002) and of course obtain soil-derived nutrients for fungal use, some of which are passed to the plant.

Two big physiological questions relate to how the fungi release P (and other soil-derived nutrients) to the plants and conversely how the plants release sugars to the fungi. The releases (efflux steps) are central to symbiotic function and are underpinned by complex molecular dialog between the partners and marked changes in cellular organization, leading to the development of the specialized fungus-plant interfaces (Genre and Bonfante 2007, Genre et al. 2008, Genre et al. 2005).

Development of colonization of roots by AM fungi is the subject of a great deal of current research, understandably focusing on plant processes and
capitalizing on an increasing number of plant mutants that block colonization at different stages.

A suite of plant genes and an increasing number of signal molecules have been identified that facilitate stages of fungal entry and development (Parniske 2008). The fungal side of the picture is less clear, but fungal identity (and hence genetic variation) certainly influences the outcome of colonization in terms of the morphology of the infection units and types of intracellular structures that are formed (Cavagnaro et al. 2001, Gao et al. 2001, Kubota et al. 2005, Smith et al. 2004). Developmental and physiological variations in outcome of the symbioses that clearly can be attributed to variations in AM fungal capabilities have been largely omitted from investigations, but future work should address this important area.

Signaling between the symbionts plays key roles in the developmental processes. Again more is known about the plant than the fungal side of the picture (Bonfante and Genre 2010, Harrison 2005, Parniske 2008), but fungal signals (or MYC factors), which operate at early stages of the colonization process, have been identified (Maillet et al. 2011). The plant often is considered as being in control of the symbiosis. This view is probably a function of our greater knowledge of the plants, emphasis on the variations in structural and physiological responses of the plant to fungal colonization and the fact that many plant species (unlike fungi) are not obligately dependent on the symbiosis for survival. The phytocentric view is also nurtured by the idea that AM symbiosis is a strategy that is suppressed
or rejected by the plants when not needed to increase P uptake (Smith and Smith 2011a, b).

In natural ecosystems, instead of heavily fertilized agriculture or pot experiments, elimination of AM colonization by the plant is not the normal situation. The organization of internal mycelia (infection units) is variable (Gallaud 1905). For many years it was thought that the variation depended on the identity of the plant, supporting the idea of plant control of development (Smith and Smith 1996). However recent investigations have shown clearly that fungal identity also plays a significant role and that different AM fungi develop in different ways in the root system of the same plant species (Cavagnaro et al. 2001, Dickson 2004, Kubota et al. 2005 and Smith et al. 2004).

Fig 1: Drawing of rhizosphere versus mycorrhizosphere. The rhizosphere (left) and mycorrhizosphere (right) of ectomycorrhizal (EM) pine seedlings.
differ dramatically from one another in plant and soil attributes adapted from Cardon and Whitbeck, 2007.

Furthermore variations in development extend to the abilities of different fungi to interact with and colonize roots of plants in which individual genes in the common symbiotic pathway have been mutated (Gao et al. 2001, Poulsen et al. 2005). Many plant-fungus combinations develop infection units of the so-called Arum type, characterized by intercellular hyphae in the cortex, from which highly branched arbuscules develop by invagination of cortical cell plasma membranes3 (Gallaud 1905).

**Fig. 2:** A mature Arum-type arbuscule of *Glomus mosseae* within a cortical cell of *Allium porrum* (leek). The arbuscule has grown from a well-developed intercellular hypha, adapted from Smith and Read, 2008.
There are therefore two types of plant-fungus interface, intercellular and intracellular, which might have different functions (Fitter 2006, Gianinazzi-Pearson et al. 1991, Helgason and Fitter 2009). They have simple intracellular hyphal coils or coils bearing arbuscule-like branches (arbusculate coils) and the intracellular interfaces must perform all intersymbiont transfer functions.

**Fig. 3:** Root colonized by endomycorrhizal fungus. Note the zone of P (or other nutrient) absorption by a non-mycorrhizal root(A) and by a mycorrhizal root (B) P phosphate ion, adapted from Saghir Khan et al., 2010.
Between these two major infection types exists a range of intermediates (Dickson 2004). Most investigations have focused on symbioses of the Arum-type and there has been little systematic investigation of developmental and physiological aspects of other forms, although limited information suggests that Paris-type arbuscular mycorrhizas have many similarities to Arum-types, both with respect to key aspects of development (Genre et al. 2008) and function (Cavagnaro et al. 2003, Karandashov et al. 2004, Smith et al. 2004). It is important that models of AM function encompass all the arrangements of interfaces that exist or explicitly distinguish among the types. Most research has been carried out on model plants.

Fungal arbuscules and hyphal coils are often described as intracellular because they grow from hyphae that penetrate the walls of root cortical cells. However these fungal structures remain in an apoplastic compartment outside the cortical cell plasma membranes, which become invaginated and often also modified as the fungal structures grow. The fungi that typically form Arum-type arbuscular mycorrhizas, and in consequence there is a tendency to assume that all colonization conforms to this type and to generalize and create models based on this assumption.

The external mycelium of AM fungal genotypes also is variable (Smith and Read 2008), with differences identified in dimensions, branching patterns and ability to colonize other plants and absorb nutrients at a distance from the roots (Drew et al. 2003, 2005; Jakobsen et al. 1992a, b; Jansa et al. 2003; Munkvold et al. 2004). However difficulties of study in soil restrict the
information available, which is unfortunate because (from a plant perspective) this mycelium plays a critical role scavenging Pi, based on several key features. The hyphal length density of the mycelium in soil (HLD, m g⁻¹ soil) normally is considerably greater than the root length density (RLD). For example Li et al. (2008b), using wheat grown in a calcareous soil with naturally occurring AM fungi (≈ 80% root length colonized), had a RLD of 0.04 m g⁻¹ dry soil and associated HLD of mycorrhizal fungi of 2.5 m g⁻¹. Hyphae (approximately 2–20 mm diam) are much narrower than roots (≈ 300 mm) and of similar diameter to root-hairs but of much greater length; this has several important consequences. Production of absorbing length (an important characteristic in uptake of immobile nutrients) by hyphae is much less expensive than for roots. Their narrow diameter also means that hyphae can grow into soil pores of considerably smaller diameters than roots are able to do and hence can exploit the P in the soil solution in a given soil volume more effectively and at lower soil moisture. In addition narrow diameter reduces the problem of depletion of P at the uptake surfaces, which is consequently much less for hyphae than for roots (Tinker 1975b). Moreover when AM plants are grown on soils with poorly available (i.e. adsorbed) P their responsiveness to colonization is higher than when grown on soils with equivalent amounts of readily available P. This effect may be due to better access to adsorbed P by AM fungal hyphae compared with roots (Bolan et al. 1987)

The possibility that AM fungal hyphae have strong capability to hydrolyze organic P is still unresolved (Joner et al. 2000). All these features, coupled with marked efficiency in translocating P from sites of absorption to transfer
sites within the root cortex, render the external mycelium an extremely efficient P uptake and delivery system.

**Mixed growth response of Mycorrhizal Colonization**

Because the external mycelium scavenges for P so effectively AM symbiosis frequently leads to higher P uptake and growth of AM plants, compared with an NM counterpart; the plants are said to show a positive mycorrhizal response. In contrast some plants show low or no positive growth responses under the same conditions; examples are varieties of barley and wheat and some prairie grasses (Hetrick et al. 1993a, b; Tawaraya 2003; Wilson and Hartnett 1998). Low positive or zero responsiveness is conventionally attributed to extensive, finely branched roots and dense and long root hairs that allow efficient P uptake in the absence of AM colonization (Baylis 1972). Responses also can be negative. Until recently it was generally thought that negative responses were abnormal and probably engendered by environmental conditions of pot experiments, particularly low light.

However it has become increasingly apparent that this is not the case and that an enormous range of growth responses from highly positive to negative can occur (see Klironomos 2003, Tawaraya 2003). For many years zero or negative responses (growth depressions) received little attention (Smith 1980). This position was reversed in an important review that focused on the diversity of plant responses to AM colonization, highlighting what then was believed to be a mutualism-parasitism continuum, based on variations in C–P trade between the symbionts (Johnson et al. 1997).
This part of the chapter expanded on the conventional ideas that when a growth depression occurred it was thought to be the result of excess drain of photosynthate (C) to the fungus. It generally has been assumed that the contribution of root P uptake directly from the rhizosphere was the same whether or not the plant was colonized.

Possible mechanisms for increased uptake of P by arbuscular mycorrhizal (AM) plants, modified from Bolan et al. (1984a). Thick arrows indicate interconversions of forms of P during an experiment, with the thick dashed arrow indicating net hydrolysis of organic P. Thin dashed arrows indicate effects of AM fungi, as suggested by Bolan et al. (1994a).

1) Extensive physical exploration of soil by fungal hyphae;
2) Higher substrate affinity of P uptake into hyphae than directly into roots;
3) combined extensive physical exploration and chemical modification of adsorbed P that speeds up soluble P release;
4) Possible fungal hydrolysis of organic P.

Accordingly when AM plants had the same or lower total P content than NM counterparts (as in a plant showing zero or negative growth response) the AM fungi were assumed to make no contribution to overall P uptake; the symbiosis was not working. The fungi were assumed to be parasites; they were cheating the plants by using a highly evolved symbiotic pathway to gain entry to the roots and taking C but delivering no nutritional benefits (Kiers and van der Heijden 2006). However investigations using radioactive P have shown these assumptions to be in error, as will be discussed in the
next section (see Jakobsen 1999; Smith et al. 2009, 2011; Smith and Smith 2011a).

Plant and Fungal nutrient uptake and transfer Pathways in AM Plants

AM plants have two pathways that can contribute to nutrient uptake from soil; direct uptake from the rhizosphere by root epidermal cells (including root hairs, when present) and the AM uptake pathway via the fungi. Non-mycorrhizal plants (noncolonized potential hosts of AM fungi or non-hosts) take up P only by the direct pathway via Pi transporters expressed in the epidermis, but both pathways may operate in an AM plant. All AM plants investigated so far, whether or not they characteristically respond positively to AM colonization, possess Pi transporters that are specifically or preferentially expressed in root cortical cells when these are colonized (see Bucher 2007, Javot et al. 2007), providing markers for the AM pathway and good circumstantial evidence for the site of P delivery via the fungal pathway and transfer to the plants. The two pathways in an AM root are potentially independent because they involve uptake from different soil locations (rhizosphere or soil several centimeters from the roots) and expression of different Pi transporters in different cell types (epidermal or cortical cells). However this does not mean that they do operate independently. Use of radioactive isotopes of P (33P and 32P) to trace the contribution of the AM pathway has shown that it can make a major contribution to plant P uptake not only when the symbiotic plants show large
increases in growth and total P but also when they do not (Smith et al. 2004, 2009; Smith and Smith 2011a).

In an extreme case *Glomus intraradices* delivered 100% of plant P to AM tomato. Such findings mean that the contribution of the direct pathway is decreased, often markedly so (see Smith et al. 2011, Smith and Smith 2011b), and highlight a highly significant role for the fungi, not only in P uptake via the AM pathway but also in reducing the contribution of the direct root pathway. As yet we do not know whether the reductions in direct uptake are always the result of fungus–plant signaling and possibly lower expression of P transporters in root epidermis or whether competition for Pi between roots and external AM mycelium also contributes by reducing the Pi concentration in the rhizosphere available for direct uptake (Smith and Smith 2011a). Investigations of the relative contributions and amounts of P absorbed via the direct root and AM pathways and discussion of possible mechanisms so far has concentrated on plants that do not show positive AM responses in growth or P uptake (Christophersen et al. 2009; Facelli et al. 2010; Grace et al. 2009a, 2009b; Poulsen et al. 2005). Increased investigation of the interplay between AM and direct pathways in positively responsive plants is necessary. In any event it is clear that the AM pathway potentially makes a large contribution to P uptake by all AM plants, regardless of their growth or P uptake responses. It is important to note the contribution of the AM pathway cannot be calculated by subtracting total P in NM plants from total P in AM plants because this calculation depends on the erroneous assumption that direct uptake in AM plants is the same as in NM plants. The errors are likely to be small in a highly responsive plant in
which total uptake is greatly increased by the symbiosis but will be large in a poorly responsive plant. There are serious gaps in our knowledge of two key steps that underpin the bidirectional transfer of nutrients on which the symbiosis is based. We know almost nothing of the mechanisms of P loss from fungus to the interfacial apoplast surrounding intracellular arbuscules or hyphal coils. Efflux must be energetically downhill (i.e. passive) and therefore inexpensive, but mechanisms may be specialized because plants and fungi expend considerable energy taking up and retaining P. Abnormally rapid efflux from the intracellular fungal structures, in itself apparently disadvantageous to the fungus, probably is required to sustain the measured overall flux of P into the plants (Smith et al. 1990, 1994, 1995). It is the price the AM fungus apparently has to pay to maintain the symbiosis. Moreover all transfers of negatively charged P forms during uptake into hyphae, long distance translocation and transfer across the symbiotic interface require charge balance to be maintained. This requires simultaneous transfer of positively charged ions (e.g. K+ or arginine) with Pi (Smith and Smith 2011a) because reverse transfer of organic C to the fungus is not necessarily an energetic burden on the plant if plant photosynthesis is sink-limited and especially if C supplied by the phloem is diverted from root growth to fungal growth, resulting in lower root : shoot ratios of AM plants, as often observed under normal light conditions. Although it has been shown that glucose is the sugar absorbed by the fungus (Shachar-Hill et al. 1995), little is known of the sites or mechanisms of efflux from the root cells and uptake by the fungus that would promote continuing C transfer. Some clues relating to sugar efflux from plant cells are beginning to be revealed. The first sugar carrier identified from plants has been cloned and characterized.
(AtSWEET1) and shown to belong to a gene family with 17 family members in Arabidopsis (Chen et al. 2010), which does not host AM fungi. AtSWEET1 is a low affinity uniporter (Km, 9 mM), capable of mediating both glucose efflux and uptake; the direction would depend on the concentration gradient across the membrane. Members of the family are important in the process of phloem loading and in delivery of sugars to developing pollen tubes and, relevant to this discussion, delivery of sugars (glucose) to bacteria and fungi that live in the leaf apoplast. In tomato the bacterial pathogen Pseudomonas syringae produces an effector molecule that activates the gene and increases sugar efflux; P syringae mutants that cannot inject the effector into host cells show compromised pathogenicity.

In rice a mutation in OsSWEET11, which interferes with pathogen-specific induction of the gene, confers resistance against bacterial blight (Xanthomonas oryzae). The fungal pathogens Botrytis cinerea and Golovinomyces cichoracearum also induce members of the AtSWEET family and are presumed to alter sugar efflux at infection sites. It is not too much of a conceptual leap to suggest that AM fungi might have similar ability to increase sugar export from root cells via induction of related root-expressed genes (see Chaudhuri et al. 2008). Uptake of hexose from the interfacial apoplast by a fungal sugar transporter (Schübler et al. 2006) could then follow. Localized intraradical expression of the fungal sugar transporter must be assumed because external mycelium does not take up sugar and therefore must lack such transporters. Such a mechanism of sugar transfer, which currently is speculative, does not depend on reciprocal
transfer of resources such as P between symbionts (i.e. it will allow for differences in C–P trade balance).

**MECHANISMS OF TRANSFER AND C AND P TRADE**

AM fungal parasitism (or cheating) might be rare. — The demonstration that the AM pathway can make a highly significant contribution to P uptake when there is no positive plant growth response means that the view that AM fungi act as parasites (or cheaters) cannot be upheld in these interactions. In all cases where delivery of P via the AM pathway using tracer has been assessed in nonresponsive or negatively responsive plants the AM pathway was (with one exception, Ravnskov and Jakobsen 1995) shown to be operating (see Smith et al. 2009). The quantitative aspects of mutualistic C–P trade almost certainly vary (e.g. with different AM fungi), but there have been few detailed investigations using techniques that actually measure the amounts of C or P transferred instead of inferring these from plant biomass and P content (Pearson and Jakobsen 1993). Implications of such variations when more than one AM fungus colonizes a root (as is normal in field situations) both for fungal and plant success are not yet clear. Excessive C drain may not be the physiological basis for lack of positive growth response.—Growth depressions have been attributed conventionally to fungal demands for organic C outweighing benefits of improved P nutrition (thought to be negligible due to inoperative AM pathway). However we now know from the studies with 32P and 33P that the AM pathway does operate but remains hidden if P transfer is based on calculations using total plant P in AM and NM plants. Furthermore large growth depressions in low P soil can occur when AM fungi colonize poorly
(low percentage of root length colonized) and would not exert a large C drain (see Grace et al. 2009a, b; Li et al. 2008a; Smith et al. 2009). It therefore has been suggested that the low plant growth and low P uptake (P deficiency) of AM plants is a function of reduced direct root P uptake that is not sufficiently compensated for by delivery via the AM pathway in the poorly colonized roots (Grace et al. 2009a, Li et al. 2008a, Smith et al. 2009). What is significant is that low colonization is sufficient to inhibit direct root uptake, making it likely that fungus-plant signaling that affects direct Pi uptake mechanisms are the basis for the reduction. At high soil P the situation might be different. Johnson (2010) has suggested that growth depressions (‘‘parasitism’’ in her terminology) may be induced when the availability of both P and N in soil is high. If large amounts of N are transferred via the AM pathway to a nonresponsive or negatively responsive plant this, like P transfer, must be hidden (not appear as increased N content in the smaller AM plants). Furthermore translocation of arginine through the fungi followed by breakdown to ammonium (NH4\(^+\)) and transfer of NH4\(^+\) across the symbiotic interface (Govindarajulu et al. 2005, Jin et al. 2005) would result in considerable loss of C as CO2 (Smith and Smith 2011a) and this might be the cause of the growth depressions compared with NM plants.

Detailed physiological investigations of this hypothesis (including use of isotopically labeled P and N to measure the amounts delivered to the plants via the AM pathway) are required. However it must be borne in mind that with high soil N and P the percentage of root length colonized is likely to be low, so that supply of C to the fungus and amounts of P (and possibly also N) transferred will be relatively low.
**Is fungus or plant in control?—**

The cause-effect sequence in mutualistic AM C–P trade has long been a subject of speculation. One possibility is that it evolved from fungal parasitism, that is the plant evolved to capture P from the fungus that was previously (in evolutionary terms) a biotrophic parasite that caused little damage to the plant. This situation is summarized.

In contrast Fitter (2006) and Helgason and Fitter (2009) have proposed a model of symbiotic P and C transfers in which the AM fungi direct localized delivery of P to the interfacial apoplast in cortical cells in the roots, which respond by transporting C to that site thus supporting fungal growth and powering ongoing, abnormally high, P delivery (FIG. 3b). It also is intriguing that the operation of the symbiosis appears to channel P preferentially through the AM uptake pathway, superficially supporting the idea that maintenance of the symbiosis depends on P delivery via this route. However it seems to us necessary to take into account evidence that signaling influencing membrane transport processes at the interfaces will involve those commodities that each of the partners lack.

The fungus will direct activities to enhancing plant efflux of C to the apoplast (as suggested above for leaf-inhabiting bacteria), while the plant will enhance release of P from the fungus. It is important to recognize that the balance of trade for C and P (usually assessed in terms of growth and/or P responses, an approach that we have shown to be over simple) is not constant but varies depending on the plant and fungus involved. In positively responsive plants, where the contribution of the AM P uptake pathway can
be realistically assessed from measurements of total plant P, considerable differences in P delivery by different fungi have been demonstrated.

The Fitter model does not account for this variability. The model (Fitter 2006, Helgason and Fitter 2009) also depends on speculation about the two processes in AM symbiotic bidirectional nutrient transfers about which least is known—C efflux from the plant and P efflux from the fungus. If it is assumed that when Fitter and Helgason (2009) say ‘‘AM fungi actively (our italics) transport phosphate across the arbuscular membrane…’’ they mean that there is fungal control of efflux from the fungus to the interfacial apoplast, not that the efflux is itself energetically demanding or active, which is unlikely given the probable cellular and apoplastic P concentrations and inside negative electrical potential.

Possible cause-effect scenarios in arbuscular mycorrhizal (AM) symbiotic C-P trade.

Initial fungus-host recognition and early colonization and numbers indicate possible sequences. Solute transfers via direct and AM pathways are shown as solid arrows and signaling as dotted arrows with queries. (a) In past evolution the biotrophic fungal parasite using C (1) became mutualistic after the plant devised a mechanism to promote P efflux (2 and 3) at the interfacial apoplast; however the scenario allows AM fungal parasitism (cheating) to continue in some cases. (b) The Helgason-Fitter (2009) scenario by which the fungus initially releases some P (fungal payment in advance (1) and the plant responds by releasing C in large quantities (2).
Before this reward the fungus presumably depends on limited release of C to the root apoplast, in excess of the needs of the root for growth. Signaling is said to be unnecessary, and ongoing AM fungal cheating should not occur. (c) Hypothetical attempt to incorporate recent research: early inhibition by the fungi of direct root P uptake (1), in response to shoot P deficiency signaling from the shoot (2) to promote P uptake via the AM pathway, possible induction (3) of sugar release by fungi (4), and P transfer from fungus to plant (5).

By discounting signaling processes that regulate C–P trade balance the Fitter model ignores the increasingly large amount of information (albeit from NM plants) that implicates sucrose not only as a substrate for plant growth but also as a signal that is involved in coordinating plant responses to P deprivation (Chiou and Lin 2011, Hammond and White 2008). The model proposes that increased sucrose delivery to roots is the result of localized increases in P supply via the fungus. However in NM plants that have been tested under P stress enhanced sucrose delivery to roots is the result of increased loading into the phloem consequent on disturbances in starch and sugar accumulation in leaves. It is important to note that phloem-delivered sucrose acts both as a substrate for fungal and root growth and as a signal that results in increased expression of (i) plant P transporters in the direct uptake pathway and (ii) secretion of organic acids and phosphatases, all of which contribute to increasing the ability of the plants to acquire P (often referred to as P starvation responses). So in AM plants it is possible that sucrose delivery to roots results from P deprivation in the leaves if inhibition of direct P uptake is rapid. As long as the AM fungi can gain access to the
sugars the symbiosis would provide a critical component of the P deprivation response, resulting in increased P uptake via the AM pathway if the plant is able to induce P efflux from the AM fungus (FIG. 3c). This is essentially a modification of what the Fitter model proposes (Fitter 2006, Helgason and Fitter 2009), taking newly proposed signaling mechanisms into account. However there are difficulties with our proposal, not least in the timing of AM colonization during plant development. Seedlings germinating in soil containing naturally occurring infective mycorrhizal propagules become colonized rapidly (as they also do in artificially inoculated plants). For example Smith and Smith (1981) observed AM fungal entry points on roots of Trifolium subterraneum at 4 d after sowing and arbuscules at 5 d; there are many other examples of colonization in young plants (see Harley and Smith 1983, p 47), which would have occurred before seed P reserves were likely to have been exhausted and P deficiency (and consequent sucrose movement to roots) developed. Effects of P supply on AM colonization are complex. Not only very high but also very low soil P are associated with low percentage of root length colonized (Abbott et al. 1984, Bolan et al. 1984b, Tinker 1975a). In the former case increases in root growth may underlie the reductions, with direct effects on fungal growth coming into play only at high soil P. Reasons for low colonization at low soil P have not been investigated, but it is possible that C-P trade is restricted by overall poor availability of P.

Attempts to manipulate C trade by shading plants and exerting C stress have given variable results, both on colonization and AM plant responsiveness. Few of the investigations have quantified P delivery using tracers. However
in a recent experiment with wheat shading reduced growth of both AM and NM plants equally (i.e. did not change growth responses to Glomus intraradices) and had no effect on percent root length colonized, although total weight of colonized roots per plant was lower in the smaller, shaded AM plants.

Of interest, the amount of P delivered to the plants via the AM pathway (shown with 32P) was similar in the two light regimes and hence the percentage of total P delivered via this route was actually higher under shade (Rebecca Stonor unpubl). This finding appears to argue against the need for high (substrate) delivery of C to maintain P delivery via the AM pathway. Much more work in this area is needed over the full range of plant responsiveness from positive to negative. As yet causes and effects of variations in C–P trade are unresolved and we still are left with the question of which comes first, C delivery to the fungus or P transfer to the plant and what signals and mechanisms underpin these nutrient transfers. So far most research on signaling and response pathways relating to P deprivation has used Arabidopsis, which is constitutively NM, or potentially AM plants that were not colonized. However the likely involvement and importance of AM symbiosis is beginning to be recognized (Branscheid et al. 2010, Chiou and Lin 2011, Gu et al. 2010, Smith et al. 2011). We have suggested that conventional NM controls (i.e. experimental prevention of AM colonization in a potentially AM plant) might induce P deficiency stress responses (Smith et al. 2011, Smith and Smith 2011a) and the increased contribution of direct P uptake in these NM plants might be part of such responses. It is to be hoped that the likelihood that AM fungal signals influence mechanisms of
direct P uptake soon will be more generally incorporated into investigations of plant P responses. It will be important to recognize that AM symbiosis is not a late add-on strategy that the plant adopts after P reserves become depleted but an integral component of normal and early root development. Hidden P uptake provides hidden benefits.—Interplay between the two interacting pathways of P uptake in AM plants can provide benefits even in plants that do not respond positively to the symbiosis by taking up extra P. Hidden P uptake may be revealed as increased responsiveness in AM plants growing in soil with poorly available P (Bolan et al. 1987). Furthermore plants with zero or negative responses exposed to arsenate (H2AsO4 2; analogous to Pi) normally have lower As/P ratios than equivalent NM plants (Smith et al. 2010). Arsenate and phosphate are taken up by the same Pi transporters in the direct root pathway so that lower activity of these transporters following AM colonization will decrease arsenate as well as P uptake. At the same time large amounts of P but little arsenate are delivered to the plants via the AM pathway, explaining lower As/P ratios and better tolerance to the toxin (Christophersen et al. 2009, Smith et al. 2010). The activity of the AM pathway of P uptake also has been shown to enhance the ability of plants to compete with NM neighbors. AM individuals of a tomato variety that shows no AM growth response when grown singly grew better in competition with a closely related mutant that does not form mycorrhizas (surrogate non-host plant); in other words the AM plants showed positive growth responses and increased P uptake when in competition but not when grown alone (Cavagnaro et al. 2004, Facelli et al. 2010). It was shown using 32P that the AM fungal pathway was active in delivering P to the AM plant at the expense of the NM mutant (Facelli et al. 2010).
The hidden AM benefits related to nutrition (P uptake, avoidance of toxins, increased competitive ability, as discussed here), together with other non-nutritional advantages such as increased plant tolerance to drought and to some diseases (Smith and Read 2008) will result in increased plant success and hence AM fungal success via continuity of C supply. Together these outcomes provide clear rationales for the selective advantage and evolutionary maintenance of AM symbioses not only in plants that grow better with AM but also in those that do not (when grown singly in pot experiments). Other ecosystem services delivered by the symbiosis, which are less obviously related to plant or fungal success, include positive effects on soil structural stability (Miller and Jastrow 2002), C sequestration in soil (Zhu and Miller 2003) and the possibility of reduced nutrient losses to the environment (Asghari and Cavagnaro 2011, Asghari et al. 2005, van der Heijden 2010). These also might have contributed to the success and persistence of the symbiosis in less direct ways. It is clear that plants have not evolved specific resistance mechanisms against AM fungal colonization similar to those against biotrophic shoot pathogens, although extent of colonization (both as a percentage of root length and per whole root system) certainly varies, dependent on many factors including fungal identity, propagule density and P supply. On the contrary the AM colonization pathway, pioneered and presumably refined by AM fungi over millions of years, has been adopted more recently by other root invading organisms particularly symbiotic N2-fixing rhizobia and Frankia and perhaps also root parasites such as Striga (Akiyama and Hayashi 2006, Bonfante and Genre 2010, Hocher et al. 2011, Maillet et al. 2011, Oldroyd et al. 2005). Despite their obligate nature AM fungal symbionts remain remarkably successful
and integral components of plant root systems, contributing to nutrient uptake in the large majority of land plants and influencing higher level processes, such as plant competition and ecosystem stability.

**Effect of AM fungi on biomass production (Certain specific examples)**

Due to the obligate biotrophy of the fungal partner and the close contact between the fungal and plant organs, extending as far as into the individual cells of the cortex, VA mycorrhiza constitutes a particularly intensive form of symbiosis. The mutualistic nature of this symbiosis points to a process of adaptation which has long been in progress in these so very different organisms. Not only the mycorrhizal fungi enjoy good conditions for their existence and development in this ecological niche, but also the plants profit from the interaction. The close association of fungal and plant structures can be assumed to cause much more far-reaching changes to the host’s metabolism than could be explained by the often-described nutrient effect. Table 4.1 lists the physiological and biochemical changes which may occur in plants when their roots are colonized by VA mycorrhizal fungi.

The question as to the range of the effects of mycorrhiza includes the question as to whether mycorrhized plants differ from non-mycorrhized plants wholly or just in part. In roots, some of these changes (e.g. respiration and DNA methylation rate) are strictly confined to those parts of the root which are colonized by VAM fungi (table 4. 2), while the parts which are
not colonized by them behave like the roots of a non-mycorrhized root system.

**Table 4.1:** Overview of changes occurring in mycorrhized plants (DUGASSA and SCHÖNBECK, 1995)

<table>
<thead>
<tr>
<th>Root:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- increased lignification</td>
</tr>
<tr>
<td>- increased activity of enzymes such as chitinase</td>
</tr>
<tr>
<td>- reduced DNA methylation</td>
</tr>
<tr>
<td>- altered root morphology</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Shoot:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- increased transpiration and photosynthetic performance</td>
</tr>
<tr>
<td>- reduced transpiration coefficient</td>
</tr>
<tr>
<td>- increased xylem formation</td>
</tr>
<tr>
<td>- reduced levels of stress metabolites</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Root and shoot:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- increased levels of nutrients, particularly of phosphorus</td>
</tr>
<tr>
<td>- altered phytohormone balance</td>
</tr>
<tr>
<td>- levels of free amino acids increased in root and reduced in shoot</td>
</tr>
<tr>
<td>- increased levels of fatty acids and sterols</td>
</tr>
<tr>
<td>- increased respiration in root, reduced respiration in shoot</td>
</tr>
</tbody>
</table>
Table 4.2: Influence of VA mycorrhizal fungi on root respiration and DNA methylation of tomato plants in divided root systems four weeks after inoculation (DUGASSA and SCHÖNBECK, 1995)

<table>
<thead>
<tr>
<th>Inoculation Mycorrhizal status</th>
<th>Root respiration</th>
<th>DNA methylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>with VAM of divided root system</td>
<td>[µmol CO2/kg fresh weight x s]</td>
<td>[%]</td>
</tr>
<tr>
<td>- non-mycorrhized</td>
<td>8.34</td>
<td>19.53</td>
</tr>
<tr>
<td>+ non-mycorrhized</td>
<td>8.39</td>
<td>18.58</td>
</tr>
<tr>
<td>+ mycorrhized</td>
<td>12.95</td>
<td>14.48</td>
</tr>
</tbody>
</table>

The effects of the symbiosis on the shoot must be interpreted as being systemic. They can, however, differ qualitatively from those in the root (see table 2). Further changes indicative of the effect of the symbiosis include an increased xylem formation, increased transpiration and photosynthetic performance, and increased levels of phytohormones, lipids and sterols (DUGASSA and SCHÖNBECK, 1995).

In the literature, an improved supply with nutrients, notably phosphorus, is often cited as the cause of the improved growth which VAM induces (GERDEMAN, 1968; BIERMANN and LINDERMANN, 1983). A similar shift in the root-shoot ratio as is observed after inoculation with VAM fungi can also occur when P supply is improved following a period of P deficiency (BASS and LAMBERS, 1988). Substantial growth improvement following inoculation with VA mycorrhizal fungi has been confirmed for cereals such
as wheat, barley and maize, for leguminous plants such as soy, lucerne and white clover as well as for numerous other plants cropped worldwide (MOSSE and HAYMAN as cited by WERNER, 1987). For oranges, increases to fourfold the previous yield were ascertained following inoculation with *Glomus fasciculatum*, and in the case of coffee, the increase following inoculation with *Gigaspora margarita* even was as high as tenfold the previous yield. For soy beans, grain yield increased just as much following inoculation with *Glomus sp*. As plant growth (shoot weight), by 11-13 grammes per square meter (Bagyaraj as cited by Werner, 1987).

In field experiments, inoculation with VA mycorrhizal fungi was observed to have growth stimulating effects on wheat (Wanisch, 1990; Land, 1990), flax (DRÜGE), maize and barley (HÖflich and Glante, 1991) as well as *Tagetes erecta* (Janhn et al., 1995).

Bohra and Anil Vyas 2012 performed a controlled pot experiment; agroforestry legume trees Acacia senegal (L.) Willd. and Acacia nilotica (L.) Willd. Ex. Del seedlings were inoculated with three different species of arbuscular mycorrhizal fungi (AMF), *Glomus deserticola*, *Glomus fasciculatum* and *Gigaspora margarita* which were isolated by extraction from the soil from different localities of western Rajasthan. Inoculation treatments under water deficiency significantly increased biomass production and nutrient uptake in both the species of Acacia as compared to non-inoculated. While correlating mycorrhizal root colonization with abiotic factors, it was observed that increase in soil pH with decrease in soil phosphorus and soil nitrogen resulted in increased percentage root
colonization as the study revealed. The most efficient response was observed by G. fasciculatum which resulted in more than two fold increase in nutrient uptake in case of A. senegal while G. deserticola responded most efficient towards A. nilotica.

Micropropagated plantlets of avocado (*Persea americana* Mill.) exhibit a very slow rate of growth during the acclimatization phase, possibly because mycorrhizae are absent. Inoculation of plantlets with the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum* (Thaxter sensu Gerd) Gerd and Trappe improved formation of a well-developed root system that was converted into a mycorrhizal system. Introduction of the mycorrhizal fungus at the time plantlets were transferred from axenic conditions to ex vitro conditions improved shoot and root growth; enhanced the shoot: root ratio; increased the concentration and/or content of N, P, and K in plant tissues; and helped plants to tolerate environmental stress at transplanting. Inclusion of soil as a component of the potting medium appeared to favor mycorrhiza formation and effectiveness. Thus, mycorrhiza formation seems to be the key factor for subsequent growth and development of micropropagated plants of avocado. (Vidal et al 1992)

Abdoulaye et al 2003 performed a greenhouse experiment was carried out in a sandy soil with a low available phosphorus to evaluate responsiveness of four *Solanum aethiopicum* cultivars to indigenous arbuscular mycorrhizal fungi. Results showed clear interaction between genetic variability of cultivars and fungal isolates on shoot biomass and on mineral status. Arbuscular mycorrhizal fungi can be ranked as *Glomus aggregatum*
Glomus mosseae > Glomus versiforme for improving yield as well as nitrogen, phosphorus, and potassium acquisition of Solanum cultivars.

Miranda et al. 2011 with the objective of determining whether arbuscular mycorrhizal (AM) colonization would alleviate salt stress on the growth of cape gooseberry plants, a saline soil (ECs of 5.65 dS m-1, available phosphorous of 48.1 mg kg-1) was inoculated with AM fungi (Mycoral®) (+AM) and compared to a non-inoculated saline soil (–AM). The open field experiment was conducted over the course of 131 days on the Marengo farm of the Universidad Nacional de Colombia (near Bogotá, 4°42' N, 74°12' W, 2543 m a.s.l., 14°C mean temperature, and 800 mm a-1 precipitation) where the plants were irrigated with water (ECs of 1.65 dS m-1) from the salt-contaminated Bogota river. Mycorrhizal dependence, AM colonization, relative field mycorrhizal dependency (RFMD100), dry matter (DM) accumulation and growth parameters (unit leaf rate [ULR], leaf area ratio [LAR] and specific leaf area [SLA]) were determined. The percentage of AM colonization was 29.7% in +AM plants, but only 12.5% in –AM plants. The RFMD100 index peaked at day 61 (42.5%) and decreased to 7.8% by day 89. Inoculation with AM fungi increased plant dry matter accumulation by 7%, especially stem DM, compared to –AM plants. Generally, growth rates were higher in the +AM plants; ULR increased more in the second half of the experiment in inoculated plants compared to non-inoculated. The mycorrhizal infection enhanced leaf area growth, which resulted in increased LAR and SLA, especially during the initial phases of the experiment.
The effect of root colonization by Glomus mosseae on the qualitative and quantitative pattern of essential oils (EO) was determined in three oregano genotypes (Origanum sp.) (Khaosaad et al. 2006). To exclude a simple P-mediated effect through mycorrhization the effect of P application to plants on the EO accumulation was also tested. In two genotypes the leaf biomass was increased through mycorrhization. Root colonization by the arbuscular mycorrhizal fungus (AMF) did not have any significant effect on the EO composition in oregano; however, in two genotypes the EO concentration significantly increased. As EO levels in P-treated plants were not enhanced, we conclude that the EO increase observed in mycorrhizal oregano plants is not due to an improved P status in mycorrhizal plants, but depends directly on the AMF–oregano plant association.
SECTION 3

Objectives

a) To study the root percent colonization in control and test plants.

b) To study the spores count in rhizosphere soil of control and test plants.

c) To study the various morphological and anatomical parameters associated with extent of AM colonization in control and test plants.

d) To estimate the biomass of root and shoot in control and test plants and to calculate the tolerance index of test plants with respect to control.

e) To correlate the tolerance index with control to establish the tolerance level of test plants.

f) To differentiate the morphological parameters of control and test plants.
SECTION 4
MATERIALS AND METHODS

Plants of *Vetvaria zizanioides*(Linn.) under natural conditions in experimental plots and screened for the presence of vesicular arbuscular mycorrhiza in fine root segments at regular intervals. Rhizosphere soil was also collected at regular intervals to investigate the distribution of VAM fungi in soil. Biomass and growth parameters of plant in all the experimental plots were noted at regular intervals.

**Collection of plant and soil samples**

Soil and plant samples each were collected from sites where Nickle contamination in soil was expected (The collection locations were described in previous chapter). Plant samples were collected carefully using hand trowel to dig the soil around the plant and the plants were pulled out carefully, ensuring that no part of the root was lost. The different plant samples were kept in different polythene bags and properly labeled. Soil samples were collected from the same point where the plant samples were uprooted. The soil samples were collected to a depth of 15cm using a soil auger. The soil samples were kept in polythene bags and labeled to avoid a mix-up of the different soil samples.

Clums of *Vetvaria zizanioides*(Linn.) procured from Central Soil Salinity And Research Institute, Karnal, Haryana and planted in prepared plots (4x4inch) of 15 kg soil capacity containing sandy soil from the different
locations in and around New Delhi, India (28.38N, 77.11E, 228 m altitude) during the winter season. The climate of Delhi is semi-arid and subtropical with the mean annual rainfall of about 650 mm. Three plots for each treatment were used for cultivating the plants. Nickle supplied in the form of soluble nickel salt dissolved in double distilled water (DDW), was mixed thoroughly in the soil. The prepared plots were placed in field conditions to expose the growing plants to natural environment. Ten plants per plot were maintained. 30-day-old plants were analyzed at 15 days interval for investigating the uptake potential. The culms of Vetvaria zizanioides (Linn.) grass with root (10 cm) and shoot (20 cm) were selected for the study. This experiment was conducted over a 12-week growth period with three treatments to Vetvaria zizanioides (McDonald S., 2006).

**Plant culture**

**Material required**

1. Rhizosphere soil from contaminated site.
2. Plastic bucket of 1-L capacity to mix rhizosphere soil and water.
3. Set of sieves for wet sieving and spore extraction from soil suspension.
4. Binocular microscope to observe AM spore morphology.
5. Pots (14x13 cm) to grow test plants.
6. Pasteurized coarse sand and vermiculite (3:1) to mix with the rhizosphere soil (1:1) as growth medium for the initiation of AM infection in Sudan grass.
7. Sudan grass seeds as test plant.
8. 5% Sodium hypochlorite solution to sterilize seeds.
9. Methylene stain (1% aquouse) for staining roots to observe AM fungal infection in the roots.
10. Microscopic glass slides and cover slips.
11. Compound microscope to observe AM fungal colonization and calculate percentage infection.

**Analysis of rhizosphere soil of test plants**

Different methods are known for the extraction of vesicular-arbuscular fungal spores from soil. They are wet sieving and decanting, floatation-adhesion, density gradient centrifugation and differential water/sucrose centrifugation techniques (Gerdemann, 1955; Ohms, 1957; Gerdemann and Nicolson, 1963; Mosse and Jones, 1968; Sutton and Barron, 1972; Furlan and Fortin, 1975; Allen et al., 1979; Mertz et al., 1979; David C. Ianson and Michael F. Allen, 1986). The efficiency of the separating techniques are limited by the texture of the soil samples used. The method used here in this study for sampling soils was wet-sieving and decanting (Gerdemann,1955; Gerdemann and Nicolson, 1963). This method has been slightly modified by Smith and Skipper (1979). The method is as follows:

In a beaker, 20 g. of air dried soil was taken to which 500 ml of water was added. The resulting suspension was stirred for about 30 minutes, followed by 5 minutes settling time. Thereafter soil solution was passed through the sieves of different mesh numbers (100, 200 and 300 in the increasing order). The contents of all the mesh numbers were washed individually and suspended in water in separate beakers. VAM fungal
spores have a strong tendency to stick to the glass surfaces, so soil solution in a beaker was gradually stirred to remove them from glass surface, and to allow them float on the surface of water. This solution was immediately poured onto the filter paper (Whatman No.1) and was spread in petridish. The spores were then observed under a dissecting binocular microscope. The spores were readily seen among the soil particles by their characteristic hyaline to coloured subtending hyphae, spore wall and size. These spores were picked up with a needle on slide. Microscopic examination was done by mounting the spores in lactophenol. Finally the slides were sealed with quick fix; since it is transparent the original colour of spore was also preserved.

Spore counting was done by taking 10 gms of soil sample. To facilitate accuracy in counting, semicircles were made on a dry filter paper and each semicircle was numbered as 1,2,3,4 and so on. The counting was done in each numbered, semicircle and noted separately. Spore count of one soil sample was recorded on the average of 3-5 samples of the respective examined under binocular microscope. Spore count is denoted in the tabular form.

**Assessment of roots for AM colonization**

**Measurements and analysis:**
Ten randomly chosen plants were harvested after an interval of 15 days after planting the tillers. Shoot dry weight was determined after drying the shoot tissue in an oven at 80°C for 48 h. Oven-dried shoot tissue was ground and sieved through a 0.5 mm sieve. Assessment of roots for AM colonization
was made at the end of the experiment by random sampling of roots. The roots were clarified and stained according to the method of *Phillips & Hayman (1970)*. All AM fungal structures (hyphae, arbuscules and vesicle) found in the roots were counted under the microscope and the extent of the colonization was estimated in terms of percentage of mycorrhizal root length.

**Estimation of percentage mycorrhizal colonization:**

Several methods have been described to quantify Vesicular-arbuscular mycorrhizal fungi in roots of several plants (*Nicolson, 1985; Sutton, 1973; Read et al., 1976; Ambler and Young, 1977; Ames and Lindermann, 1977; Davis et al., 1979)*.

For the present study the mycorrhizal status of plants were analyzed by:

(a) For rough calculation of percentage of infection, the technique of *Nicolson (1985)* was employed. The number of root segments infected by VAM fungi and uninfected were recorded in this technique and the percentage root infection was then determined as follows:

\[
\% \text{ root infection} = \frac{\text{Number of infected segments}}{\text{Total No. of segments infected}} \times 100
\]

For obtaining accuracy, in percentage mycorrhizal root colonization, the maximum limit of number of randomly selected segments were kept as 50. These infection segments were then used for light microscopic studies to reveal the VAM fungal structures formed into the root tissues. (b)
Quantification of the VA mycorrhizal infection was done by using the morphometric technique described by Toth and Toth (1982).

In this method a grid system of dots or intersecting lines were superimposed on the image of the specimen and the number of points lying over the infected cells of the specimen was counted. The total number of points covering the whole area of the specimen was also noted down.

In morphometric literature, with a symbol like Pp, the upper letter represents the number of test points lying over the structure in question and the subscript represents the number of points lying over the test area. Therefore,

\[ P_{fungus}/p_{fungus} = P_p \text{ for fungus in plant} \]

For more accurate measurements, care was taken to make the squashes as thin as possible because morphometric formulae are accurate only for two dimensional views.

The analysis of infected root segments was then done in detail in the manner as described below:

I. **External spread of VAM fungi:**
   1. Hyphae running parallel to the root surface,
   2. Hyphae running radially across, the root surface,
   3. Hyphae running in longitudinal, parallel and spiral manner on the root surface,
   4. Presence or absence of extramatrical vesicles.
II. Entry of VAM fungi into the root tissue:
   1. Direct Entry
      (a) Number of entry points per mm. of root tissue.
      (b) Thickness of penetrating hyphae.
   2. Indirect Entry
      (a) Number of appressoria per mm. of root tissue and shape of appressorium
      (b) Branching of infection hyphae.

Intramatrical hyphal network:
   1. Spread of infection hyphae internally
      (a) Hyphae running parallel to the root tissue
      (b) Hyphae running readily
      (c) Hyphae running in all directions
      (d) Diameter of hyphae in the cortex
   2. Infection hyphae forming secondary appressorium or appressoria; that develop secondary infection hyphae respectively, which then reach either upto the inner cortex only upto the stellar region also.
   3. Presence or absence of
      (a) Coiled hyphae (intercellular or intracellular)
      (b) Looped hyphae (intercellular or intracellular)
      (c) Projections on internal hyphae.

III. Formation of arbuscules:
   1. Presence or absence
   2. Diameter of arbuscular trunk
3. Shape and size of arbuscules
4. Arbuscular abundance (no./mm infected root)
5. Proportion of cell occupied by the arbuscule, expressed as %
arbuscular area i.e.
\[
\% \text{ arbuscular area} = \frac{\text{Area of the arbuscule}}{\text{Area of the cell}} \times 100
\]

IV. Formation of intramatrical spores of VAM fungi

Preparation of root segments for anatomical studies:
Plants were harvested at regular intervals from experimental plots. Particularly care was taken in selecting the age of plants. Roots were freed from adhering soil particles and debris by gentle washing and special care was taken, not to break the rootlets which are generally heavily attacked by VAM fungi. Endophyte invasion into the root tissue was demonstrated by clearing and staining of roots. Several methods employed initially were as follows:

1. Bevege (1968), in this technique the whole rootlets were autoclaved for 10 minutes at 12 lb/in2 in 1N KOH followed by dipping in 3% sodium hypochlorite solution acidified with few drops of 5N HCL for few minutes, staining was done in cotton blue prepared in Lactophenol. Since staining was not proper, the technique proved to be failure.

2. Hayman (1970) proposed another technique in which roots were stained in 0.1% trypan blue in lactophenol by boiling. In this technique too, due to the lack of clearing solution, no contrast was seen in the root tissue.
3. In another technique roots were boiled in cotton blue and sudan IV in lactophenol (Nicolson, 1959).

4. Gerdemann (1955) cleared the roots by boiling in 0.01% acid fuchsin in saturated chloral hydrate. However an improved technique of clearing and staining was described by Phillips and Hayman (1970), that proved to be the most successful one. Moreover in this study a modified Phillips and Hayman’s (1970) technique was used:

The whole roots were cut into small pieces of approximate 1 cm length. These root segments were floated in water in petridishes and number of segments varying from 25 to 50 depending on the size of the samples were selected randomly. These were then cleared in 10-20% KOH solution. 20% solution was used in case of hard tissues and tissues were left in the clearing solution from one to several days depending on their thickness. Soft roots were generally cut only after staining.

Pigmented roots were kept in H2O2 for one day for bleaching and then kept in 10-20% KOH for two days. In both the cases, after the initial clearing, root segments/whole roots were transferred to 1% trypan blue for staining for two days. Roots were mounted in Lactophenol. Photomicrographs were prepared subsequently.

**Experimental setup**

The experiment was conducted in 3 micro-plots of 10 m² area each. The top soil (up to 30 cm depth) was fumigated twice with 0.1% formaldehyde at an
interval of 15 days. Then the soil was allowed to dry and the fumigant was dissipated. *Vetiver zizanoides* was grown in three types of experimental plots containing sterilized soil with a known quantity of Ni salts as present in polluted soils.

**Control** without any kind of AM spores + Normal non polluted garden soil

(i) AM inoculum produced from AM spores collected from contaminated soils + test plants + Ni contaminated soil.

(ii) AM inoculums reduced from AM spores collected from normal garden soil + test plants + Nickle contaminated soil.

(iii) No Am spores + Test plants + Nickle contaminated soil.

Clums (Plate 4.1) rate was kept uniform for all treatments and when clumps were 15 days old, thinning was done to maintain spacing of 10 cm between the plants and 20 cm within the rows. The plants were allowed to grow and no fertilizer or pesticide was added to the soil during the course of the experiment. Weeding was done mechanically at regular intervals and plots were irrigated with tap water.

P1 (Plot 1) – Nickle contaminated soil+ AM fungi spores inoculum produced from spores obtained from Ni contaminated soil + Vetiver test plants.

P2 (Plot2) – Nickle contaminated soil + AM fungi spores inoculum produced from spores obtained from normal garden soil + Vetiver plant

P3 (plot 3)- Nickle contaminated soil + vetiver plants without AM fungal spores.
Control – normal non contaminated garden soil without AM spores + Vetiver plants for reference point for the study of Tolerance Index.

The morphological parameters like root length (RL), shoot length (SL), fresh weight (FW), dry weight (DW), content were recorded at regular intervals i.e. every 15 days till harvesting of experimental plant.

**Morphological or Growth Parameters:**

**Selection of the plant species:**

Vetiver (*Vetiveria zizanioides (L.) Nash*) was selected for the study because of its economical importance and easily marketable, healthy and uniform size of vetiver culm was selected (Plate 4.1). The Vetiver (*V. zizanioides*) was collected from the central soil salinity and research institute, Karnal, Haryana. The culms of vertiver was planted with 20 cm shoot and 10 cm root length in the plots of 2 feet height and 1 feet containing garden soil. Appropriate controls were maintained. After plantation, proper irrigation was done once in two days. Weeding was carried out at regular intervals. The morphological parameters like root length (RL), shoot length (SL), fresh weight (FW), dry weight (DW), were recorded at regular intervals i.e. every 15 days after planting the experimental plant.

In order to determine morphological effects of lead contamination, as well as the effects Ni contamination may have on biomass production, plants were harvested at regular intervals for qualitative analysis. Leaf colour, root colour, leaf height, and overall appearance of the plants were examined. The
total number of roots; number of developed roots, defined as the number of roots greater than 5 cm in length; and the number of offshoot stems were recorded from a representative sample of each treatment.

**Plant Growth Analysis**

**Biomass accumulation**-
The plants were harvested and washed by deionized water followed by proper blotting between filter papers. Plants were dried separately in a hot air oven at 65°C ± 2°C for 72 h. The samples were weighed on an electronic top pan balance (Sartorius BL-210S, Germany) so as to obtain the biomass accumulation, which was expressed in g per plant.

**Tolerance index** -
Tolerance Index (TI) is calculated as the mean weight (biomass) of a plant grown in the presence of a metal divided by the mean weight of a control (Baker et al. 1994).

Tolerance Index was expressed as

\[
\text{Tolerance Index (TI)} = \frac{\text{Biomass of the treated plants (g plant}^{-1})}{\text{Biomass of the control plants (g plant-1)}}
\]

TI values greater than 1 reflect a net increase in biomass and suggest that plants have developed tolerance, whereas TI values lower than 1 indicate a net decrease in biomass and a stressed condition of plants. TI values equal-to 1 indicate no difference relative to non-HM control treatments—(Wilkins 1957, 1978).
Plan of work

Plot P1, P2, and P3 are prepared with their respective soils (as discussed in material and method) without adding any kind of fertilizer. A control was also kept with non contaminated soil. The soil in these plots was fumigated using 3% formaldehyde twice in two weeks. After fumigation the soil was kept open for another week with occasionally turning it so that the traces of formalin are volatilized. When there is no smell of formalin then the soil is ready for inoculation and plantation. Fumigation was done to ensure that if there are already existing AM spores in the soil then they are killed. Before inoculation the soil was again tested for the complete removal of already existing AM spores.

Now in these fumigated soils the plantation of vetiver clumps was done. Before plantation the clumps were thoroughly washed with double distilled water so as to remove any soil particle adhering to the roots just to avoid any AM spore contamination from outside. The roots were also screened for the presence of colonization but no plant was found to have more than negligible colonization.

Inoculation and plantings of clumps was done simultaneously. One Kg of spore inoculum with nearly 100 spores per gm of inoculums was used for 100 kg’s of soil. The plants were planted in rows with appropriate gaps and watered carefully every alternate day.

The plants were gown for one season per year in all plots and all parameters were tested at an interval of 15 days. At maturity all plants were uprooted
and analyzed again for nickel accumulation and other factors. The soil in the pots was again fumigated as described above in this chapter so as to assure that there are no AM spores left behind before the sowing for next year is done.

In the consecutive year again the application of similar inoculation in test plants and sowing of vetiver in all plants was done and repeated for the consecutive third year also. At the beginning of sowing and then after every 15 days and at the end of the crop biochemical analysis was done to find out phytoremediation potential of vetiver and to find the role of AM fungi in uptake of heavy metals. (will be discussed in the next chapter.)

All parameters as discussed in material and method were taken into account and statically tested for improved uptake. All readings were taken at an interval of 15 days.
SECTION 5
Observation and results

Root percent colonization and spore count- (table 4.3, 4.4 and 4.5, Fig. 4.1, 4.2 and 4.3, Plate 4.2)

Root percent colonization in control and test plants –
Root percent colonization was calculated by the formulae described in materials and method. It is calculated at the interval of 15 days by taking roots from at least 5 different plants per treatment from different corners of the Plot.

Ist year of Growth -- In Ist yr of growth the maximum colonization was observed in plot P1 as 80% when the plants were 90 days old. The P1 contains the AM fungal inocula obtained from spores collected from contaminated soil. (Table 4.3, Fig 4.1a, Plate 4.7)

In P2 where the AM fungal inocula was prepared from spores extracted from normal garden soil the percent root colonization was slightly less as compared to P1 i.e. 72% after 90 days of growth. (Table 4.3, Fig 4.1a, Plate 4.8)

In P3 where no inoculum is added yet the soil is contaminated in Ist year of growth no colonization was reported. Comparing all the above treatments with control where normal garden soil was used with no AM spores ideally
no colonization was expected yet a negligible colonization of 18% was observed after 90 days of growth. (Table 4.3, Fig 4.1a)

Spore count in rhizosphere soil per 5 grams of soil in 1st year of growth – The spore count in rhizosphere soil was also done at the interval of 15 days till the plants are 90 day old. Rhizosphere soil of the same plants was collected which were used for studying root percent colonization then mean was taken to express the spore count. It was observed that in P1 and P2 (the only plots which contain AM spores) initially at the start have large no. of spores i.e. more than 100 due to external addition of AM inoculums whereas P3 and control have zero spores at the beginning since the soils were fumigated to ensure that if any spore is naturally present should be killed and no inoculum was added in the P3 and control. But as the root % colonization increases no. of spores decreased till almost 60 days of growth or till the roots of P1 and P2 gained nearly 70% of colonization. Thereafter P1 showed 50 number of spores at the 90th day of growth and P2 reached to 42 spores at 90th day of growth after a sharp decrease in spore count. On the other hand P3 and control showed only negligible number of AM spores since no inoculum was added and naturally occurring spores were killed by fumigation. (Table 4.3 Fig 4.1b)

IInd year of growth –
Root percent colonization in 2nd year of growth reached up to 89% in P1 and 84% in P2 at the end of 90 days of growth. Whereas as observed in 1st year of growth the treatment which do not receive AM fungal inoculm (P3 and
control) showed negligible root % colonization. (Table 4.4, Fig. 4.2a and plate 4.7 and 4.8)

Spore count also showed the same trend as shown in 1st year of growth i.e. it first decreased with increase in root percent colonization and then started increasing after the roots attained almost 70% of colonization and reached up to 78 in P1 and 68 in P2. Control and P3 again shows negligible number of spores at any time of growth. (Table 4.4 and Fig 4.2b)

**IIIrd year of Growth**

Plants growing in third year in the same plots showed a sharp and sudden increase in root percent colonization right from the 15th day of growth and reached up to 100% in P1 and 98% in P2. In control and P3 again only negligible root percent colonization was observed i.e. 18 and 10% respectively. (Table 4.5, Fig. 4.3a, plate 4.7 and 4.8)

Spore count also showed the same trend as shown in previous years i.e. first the spore count decreased with increase in root percent colonization and then increased and reached up to 76 in P1 and 73 in P2 and again remain negligible or below 15 in P3 and control. (Table 4.5, Fig 4.3b)

**Comparison and Quantification of spread of colonization / infection internally and externally in roots of Plants of different treatments.** (Table 4.6, Plate 4.9 to 4.15)

1. **EXTERNAL SPREAD OF AM FUNGI**
   a) Hyphae running parallel to the root surface.
This was observed after the AM spores germinate on the surface of root and develops external hyphae first. Control and P3 showed almost negligible (Only 2\textsuperscript{nd} in 3\textsuperscript{rd} yr of growth in control otherwise nil) parallel external hyphae. Plots P1 and P2 showed tremendous external hyphae in all three years but maximum external hyphae were observed in 3\textsuperscript{rd} year of growth i.e. 37 in P1 and 29 in P2. (Table 4.6, Plate 4.9)

b) Hyphae running radially across the root surface.

There was extensive network of radially running hyphae in P1 and P2 treatments and showed maximum number only in 3\textsuperscript{rd} year of growth reaching up to 36 and 29 at one point of time in P1 and P2 respectively. Control and P3 showed negligible radial hyphae. (Table 4.6, Plate 4.9)

c) Hyphae running longitudinal, parallel and spiral manner on root –

This also follows the same trend as the above two parameters. P1 and P2 showed up to 12 and 10 such external hyphae respectively. P3 and control showed negligible hyphae. (Table 4.6, Plate 4.9)

d) Presence and absence of extrametrical hyphae –

Extrametrical hyphae were only observed in Plot P1 and P2. In P3 and control extrametrical hyphae were absent. (Table 4.6, Plate 4.9)

2 ENTRY OF AM FUNGI INTO ROOT TISSUE

a) Direct entry
Direct entry of am hypae was observed in all treatments during all three years of study but in plot 3 the direct entry was only observed in 3\textsuperscript{rd} year of study and not in 1\textsuperscript{st} and 2\textsuperscript{nd} year. (Table 4.6, Plate 4.10)

b) **Number of entry points:**

Maximum number of entry points were observed in plot 1 and plot 2. The entry points in these plots vary between 4 to 10 but maximum entry points in these plots at any point of time were observed only in 3\textsuperscript{rd} year of growth. The entry points were maximum up to 2 in control and absent in 1\textsuperscript{st} and 2\textsuperscript{nd} yr of growth and only 1 in 3\textsuperscript{rd} year of growth. (Table 4.6, Plate 4.9)

c) **Thickness of penetrating hyphae:**

Thickness of the penetrating hyphae was more than double (4.7 micrometer) as compared to control (2.1 micrometer) and the thickness of the hyphae in plot 2 was also double then the control but only in 3\textsuperscript{rd} year of growth. Thickness could not be taken in plot 3. (Table 4.6, Plate 4.9)

d) **Indirect entry** –

Indirect entry pattern followed the same trend as was observed in direct entry i.e. indirect entry of AM hypae was observed in all treatments during all three years of study but in plot 3 the direct entry was only observed in 3\textsuperscript{rd} year of study and not in 1\textsuperscript{st} and 2\textsuperscript{nd} year. (Table 4.6)

i) **Shape and number of appressoria per mm of root tissue**

Number of appresoria varied from three to five in plot 1 with shapes ranging from swollen, flat, elliptical and compressed.
In plot 2 the number of appresoria varied from 2, 1 and 5 per mm of root length in 1st, 2nd and 3rd year of growth respectively. The shape in plot 2 ranged from swollen to flat and compressed. In control on an average only one appresoria present at any time of study and the shape appeared only swollen. In plot 3 none were observed in 1st and 2nd year of growth and only 1 flat appresoria was observed per mm of root length in 3rd year of study. (Table 4.6, Plate 4.11)

ii) Branching of infection hyphae

Branching of infection hyphae was not observed in control and plot no. 3. In plot 1 in extensive branching was observed in 2nd and 3rd year of study and nominal branching was observed in 1st year and in all three years of study in plot 2. (Table 4.6, Plate 4.11)

3) INTRAMETRICAL HYPHAL NETWORK –

a) Spread of internal hyphae internally

i) Hyphae running parallel to the root tissue

Parallel hyphae was seen in all the treatments including control except 1st and 2nd year of plot P3. (Table 4.6 Plate 4.11)

ii) Hyphae running radially

Radial hyphae was observed only in plot P1 and P2 and in the 3rd year of growth of plants in control. (Table 4.6, Plate 4.11)

ii) Secondary appresoria

Secondary appresoria was observed only in 2nd and 3rd year plants of Plot P1 and 3rd year of plot P3. (Table 4.6, Plate 4.11b)
4) PRESENCE AND ABSENCE OF –

a) Coiled Hyphae –
Coiled hyphae was found to be present in 3rd year plants of control, all years in plot P1 and P2 and none in P3. *(Table 4.6, Plate 4.15a)*

i) Looping hyphae-
Looped hyphae was observed only in plot P1 all years and plot 2 2nd and 3rd year. *(Table 4.6, Plate 4.15a)*

j) Projection in Internal hyphae-
It followed the same trend as was looped hyphae.

5) FORMATION OF ARBUSCULES

a) Present or absent –
arbuscules were clearly and well formed in 3rd year plants of control and all years in Plot 1 and 2. *(Table 4.6, Plate 4.11a)*

b) Size of arbuscules-
Maximum size was observed in Plot P1 i.e. up to 18 micrometer and minimum in control up to 6 – 7 micrometer. *(Table 4.6, Plate 4.11a)*

c) Arbuscular Abundance-
Maximum number of arbuscules per mm of infected root was 25 in plot 1 (3rd year) and 17 in plot 2. They are completely absent in plot 3. *(Table 4.6)*

d) % arbuscular area-
Maximum percent arbuscular are was observer in plot 1 i.e.42% followed by 36% in plot 2. (Table 4.6)

6) FORMATION OF INTRAMATRICAL VESICLES
They are seen in 2\textsuperscript{nd} and 3\textsuperscript{rd} year of growth of plot 1 and in 3\textsuperscript{rd} year in plot P2. (Table 4.6, Plate 4.12 and 4.13)

BIOMASS ACCUMULATION
Here the findings of biomass accumulation were in contrast to the observations of root percent colonization.

i) It was found that in 1\textsuperscript{st} year of growth the biomass of plants in Plot p1 and P2 was much lower than the control and most of the plants in Plot p3 were also very week with very low biomass. (Table 4.7, fig. 4.4a, Plate 4.2 and 4.4)

ii) All the plants in plot P1, P2, p3 showed stunted growth and chlorosis yet the root biomass in plot P1 and p2 was reasonably high then the shoot biomass but much less the control. (Table 4.7, Fig 4.4a, Plate 4.2 and 4.4)

iii) The tolerance index for plant T1 reached only up to 0.64 and for plot P2 reached up to 0.56 and for plot P3 much below the normal i.e. 0.26 only as compare to control. (Table 4.7, graph 4.4b)

iv) In 2\textsuperscript{nd} year of growth plot P1 and P2 showed better growth as compared to the 1\textsuperscript{st} year plants. In plot P1 the root biomass and shoot biomass reached very close to control plants after 90 days of growth. Plants in Plot P2 also showed better growth as compared
to 1st year plants but the growth was still incomparable with control plants. Plot p3 plants still lag behind a lot as compared to control. (Table 4.8, Fig 4.5a Plate 4.3 and 4.5b)

v) All the plots except control still showed chlorosis of varied degree. Maximum chlorosis was in plot P3 with stunted growth. Minimum was in plot P1 and the growth was normal. (Table 4.8, fig 4.5a,Plate 4.3 and 4.5b)

vi) The tolerance Index (TI) of plot P1 reached very close to the control after 90 days of growth i.e. 0.96. Whereas TI of Plot P2 also increased as compared to 1st year of growth but still lags behind that of control plants. It stands at 0.87 after 90 days of growth. The Plot P3 was way behind the growth as compared to control plants. It only achieved TI of 0.40 as compared to control. (Table 4.8, Fig. 4.5b)

vii) During 3rd year of study the Plants in P1 and P2 slightly outreached the growth in terms of biomass as compared to control. Root and shoot biomass were slightly more as compared to control. But in plot p3 the growth was less then control but reached very close to control. (Table 4.9, Fig. 4.6a, Plate 4.3 and 4.6a,b,c)

viii) The features like chlorosis and stunted growth were missing in this year of growth. (Plate 4.3 and 4.6a,b,c)

ix) The tolerance index of plot P1 and P2 was more than control 1.02 and 1.01 respectively. The Ti of Plot p3 was still much lower than control as well as of that of Plot P1 and P2. (Table 4.9, Fig. 4.6b)
SECTION 6
Discussion

Total Dry Matter Production
The first prerequisite for higher yields in plants is an increase in biomass production in terms of dry matter. Carbon compounds account for 80%–90% of the total dry matter produced by plant. Larger source size and improved photosynthetic process was found to be the basis for the building up of organic substances and dry matter production. In a study conducted on Brassica juncea to evaluate the Ni accumulation and toxicity in relation to biomass production, it was found that 100 lM Ni decreased dry mass of the plants (Alam et al. 2007). The other important characteristics determining total dry matter production is ratio of shoot to root mass. It was revealed that at low concentrations of Cd2? (10 and 50 lM) shoot to root ratio increased in maize seedlings (Baccouch et al. 2001). The important parameters contributing to total dry matter production are fresh and dry mass. According to Bashmakov et al. (2006), 21 day old maize plants showed significant loss of fresh mass at even 10 lM Cd. Moreover, an increase in Ni concentration caused almost a linear decrease in shoot and root fresh and dry mass of plants. The water content both of root and shoot significantly decreased at 0.1 mM and lM of Cd2?, that possibly is the main reason assigned for the loss of fresh and dry mass of the maize seedlings.

Vesicular-arbuscular mycorrhizae were of general occurrence in all families except Utricaceae, Casuarinaceae, Nyctaginaceae, Portulaceae,
Caryophyllaceae, Amaranthaceae, Chenopodiaceae, Oleaceae, Zygophyllaceae, Tamaricaceae and Euphorbiaceae (Khan, 1973).

Nicholson (1967), in his review emphasized that this type of mycorrhiza, although designated as endotrophic, was composed of a two phase mycelia system: an internal mycelium within the cortex of a mycorrhizal reet and an external mycelium in the silwhich varies considerably in extent but which may be very extensive in some cases, even obscuring the root. However well-defined pseudoparenchymatous sheath around the roots is never formed in case of vesicular-arbuscular mycorrhizae.

Structure produced by VA fungi within host roots include a hyphal system contiguous, through initial penetration points, with a hyphal net work extending into the soil, short lived intracellular arbuscules generally thought to function in nutrient transfer between the symbionts; and enlarged intercalary or terminal vesicles that appear to function as endophytic storage organs. There are many reports on the anatomy of the host and endophyte association (Fontana et al., 1978; Sward, 1978; Abott and Roboson, 1979; Abott, 1982; Carling and Brown, 1982; Graham et al., 1982; Sanders and Shieith, 1983).

Development of Infection:
Details of the infection process have been studied chiefly using spores or infected segments of root as inoculums, either in axenic culture in agar (Mosse and Phillips, 1971; Mosse and Heper, 1975; Heper, 1981) on slides buried in soil (Powell, 1976), production of entry points has also been
studied in pot experiments (Carling et al., 1979; S. E. Smith and Bowen, 1979; F.A. Smith and S.E. Smith, 1981; S.E. Smith and Walker, 1981) using sequential harvests, which do not, however, permit continual observation of the same infection.

When spores are used as inoculum, germination is followed by considerable growth of one or several germtubes, so that a simple mycelium in which total length of hyphae of a few centimetres is produced. Growth is sometimes increased if susceptible roots are present, so that it was at first thought that exudates from the roots might provide substrates for hyphal growth after the reserves in the spores had been used up. The role of root exudates in the development of infection is still receiving considerable attention. In spite of the increased mycelia growth in the presence of roots, hyphae may not appear to make directional growth towards roots until they are very close to them, i.e. within a few millimetres (Mosse and Heppar, 1975; Powell, 1976).

Formation of an appressorium on the root epidermis is normally followed rapidly by penetration of the epidermal and cortical cells by hyphae and development of typical mycorrhizal structures within the root. Infection peg push into the cell wall. The later buldges round the hyphae and, in cortical cells, becomes much thinner (Cox and Sanders, 1974). This buldging implies the exertion of pressure by the growing hyphae and a degree of extensibility, existent or induced, in cell wall. Whether enzyme production is also involved is not known, but it seems unlikely that the hyphae can generate much hydrolytic activity in view of the poor saprophytic ability of
the fungi concerned. Nevertheless, changes in the middle lamella, as seen by electron microscopy (Kinden and Brown, 1975b), when the intercellular spaces are colonized by hyphae, might be thought to give some credence to the suggestions that fungal enzymes may be important.

**Relationship in spore count & % root colonization in plot 2**

It was observed that there was steady % root colonization from 1\textsuperscript{st} year to 3\textsuperscript{rd} year in Plot 1 and 2. It was observed that the spore count and % root colonization are inversely proportional from 15 DAS, 60 DAS and 90 DAS every year (1\textsuperscript{st} yr, 2\textsuperscript{nd} yr and 3\textsuperscript{rd} yr). This may be because soil of the plot was inoculated by the spores were first germinated to produce root colonization so as colonization increased the spore count decreased but as colonization reached to an optimum level the vesicules made stat getting converted into spores and then the spore count increased. So from the above mentioned observation it was concluded that there is correlation between level of mycorrhizal infection in the roots and the spore counts of the soils around them. Above data showed that the more is the number of spores in the rhizosphere soil, the more is the degree of colonization.

**Spore count and its relation with % colonization in plot 1(Contaminated soil with inoculum from spores extracted from contaminated soils of various regions)**

Roots of vetiver were taken at regular interval (15 days) and analyzed for % root infection change & spore count changes were observed every year on 15 DAS(Initial), 45 DAS,90 DAS (maturity).
NOTE: The result of this investigation clearly show that all the experimental plant species examined is mycorrhizal, their type being Vesicular-arbuscular and it does not show any other type of mycorrhiza. This result is in accordance with the detailed survey of Janse (1896) and Johnston (1949). During the present investigation it was found that the degree of AM formation and no of spores varied in all the experimental plots studied. The colonization was lowest in plot 1 in all the years because there were no AMF inoculums spores were provided to the soil and the plot 1 was used as control plot. It was observed that colonization and spore number were increased in plot 2 in all the years at each DAS studied because the soil of the plot is inoculated with healthy spores from non contaminated soil.

As compared to plot no. 1 there were less no of spores and % root colonization in plot 2 in all the three years. This may be because the spores extracted from contaminated soil were better adapted for survival and causing infection in root as compared to spores extracted from normal non contaminated garden soil. The spores from non-contaminated soil showed statistically insignificant yet less percent colonization and other parameters as compared to spores extracted from contaminated soil. Moreover no sporocarp was extracted from the contaminated soil. The presence of unhealthy spores in contaminated soil can be attributed to two reasons

i) Presence of lot of heavy metal contamination

ii) The vegetation cover in the contaminated sites was very scarce and for the proliferation of AM Fungi roots of plants is a necessity as they are obligate parasites.

iii) Though the spores extracted from contaminated soil were not very healthy but they were well adapted for the survival in
contaminated soil and on getting proper conditions and compatible host they survived better than the spores isolated from contaminated soil. Despite the high level of contamination found in the Yamuna soil the spore morphology, spore count and % colonization was comparable with non contaminated garden soil as there was lot of cultivated and non cultivated vegetation ion the Yamuna region. Other workers also showed similar results.

AM is prevalent in the field crops under optimal conditions (Strzemska, 1975; Hayman et al., 1976; Jensen and Jacobson, 1980; Jacobson and Nielson, 1983). The present study confirms formation of similar arbuscular mycorrhizae in Vetiver zizanioidis under the tropical soil condition in India. Nearly all plant grown in all three experimental plots showed mycorrhizal infection ranging from 5% to 85% of their fine root length .This is in accordance with the results obtained by Jagpal and Mukerji (1987).

The endomycorrhizal colononization results from three simultaneous processes viz., root growth, formation of fungal entry points on the root surface and growth of fungal hyphae along the length of the interior of root (Smith and Walker, 1981). Some plants become heavily colonized with the VA endomycorrhizae e.g. maize and onion; others become moderately infected under same conditions e.g. tomato (Hayman et al., 1976) and yet others usually develop little infection e.g. rye grass. The host plant itself affects successful entry by the host endophytes. These endophytes spread much faster in some plants than in others and thus their final colonization
levels vary considerably in different hosts. But even within optimum host-endophyte combinations, 100% infection is never achieved, for example, strongly mycorrhizal plants like onion, grown in pots, often reached a maximum of 80% of the total root length colonized (Furlan and Fortin, 1973). In the present study Sorghum bicolour showed the total maximum root length colonization of 98%.

Infected root segments, infective propagules and spores isolated from open pot cultures of AM fungi inoculated plants have been the usual source of AM inoculum for commercial purpose (Sylvia et al., 1993). Spores of AM are the important component of inoculum and are more suitable for large scale production as well as for biochemical and molecular investigations of the AM symbiosis (Thompson, 1987).

Higher level of spore density in the AM inoculum results in maximum sporulation and spore germination which paves way for the massive development of hyphae and root infection by arbuscular mycorrhizal fungi compared to the lower spore density levels and un inoculated control. Enhanced arbuscular mycorrhizal infection by higher spore density had ultimately resulted in increased root surface area which recorded a maximum root volume at 5 to 6 and 10 to 12 spores g-1 of AM inoculum application. The results clearly indicated that AM colonization and sporulation was better in p1 as compared to p2 with higher growth of host plant.
Several authors have discussed the production and colonization of AM fungi. Hung and Sylvia et al. (1993) reported that colonized roots and viable spores can serve as infective AM inocula. Nopamornbodi et al. (1987) applied 20 AM spores in 10 ml water suspension under each seed of soybean and obtained increased yield under field condition. Since large scale production of spore is difficult considering 10 spores g⁻¹ of inocula which requires, inocula in kgs involves sieving the mother inoculum in large quantity to prepare the AM inoculum with desired number of spore density for inoculating field sown crop and is laborious (Douds and Schneck, 1990).

Recent study illustrates that a spore count of 322 per 100 g of AM inoculum resulted in 80 per cent root colonization in maize at field conditions (Doud et al., 2005). Additionally, Douds et al. (2010) reported that incorporation of 75 g of inoculum containing 795 mean numbers of spores of G. mosseae at the time of preparation of nursery bags of size 21 cm diameter x 13 cm height with soil as medium for plant growth was able to enhance the root length and shoot dry weight of black pepper plants.

In the present study the Sudan grass was used for inoculum production which is susceptible to mycorrhizal infection but to different degrees. As the time advanced the intensity of infection increased. At all sampling intervals soil samples from Sudan grass contained significantly greater number of Glomus species spores. One might conclude that increased sporulation in S. bicolor is due to increased root growth, but no such correlation was observed in this study. The genus Glomus is the dominant mycospecies in the garden (rhizosphere soil) collected from different areas of Delhi, the possible
reasons for the predominance of *Glomus sp.* are that spores of *Glomus* species have different temperature and pH preferences for germination (Wang *et al.* 1997) and *Acaulospora species* are often associated with acidic soils (*Morton 1986; Abbott and Robson 1991*). The results obtained from the study suggests that the colonization percentage and number of AM spores differ with different type of soils used in the study. The soil collected from Yamuna bank has enough number of spores while the soil collected from shastri park has no spores and reason for that is the soil is contaminated with electronic waste i.e is one of the soil contamination source. This confirms that the soils of Delhi region are colonized by arbuscular mycorrhizal fungi. It is also apparent that rainy season may be considered as the best season for the propagation of plants by the application of AMF as bio-inoculant.

In the present study, for a particular plant species, root colonization, spore density were higher in non-contaminated soils (Yamuna soil) compared to metal contaminated soils (Shastri park soil).

**AM status associated with the hyper accumulator**
To date, there are >160 species of AM fungi identified. Based on the morphological and molecular identification methods, all AM fungi were divided into 12 genera (*Acaulospora, Ambispora, Archaeospora, Diversispora, Entrophospora, Kuklospora, Geosiphon, Gigaspora, Glomus, Intraspora, Paraglomus, Scutellospora*) (*Morton and Redecher 2001; Schüßler *et al.* 2001; Schüßler 2002; Sieverding and Oehl 2006; Walker *et al* 2007).
In the present study, 6 AM species belonging to 2 genera associated with (*Sorghum bicolor* L *Moench* var *sudanense*) were identified. It is highly likely that the high AM fungal species found was due to the collection of soil samples from various habitats. Predominance of the genus *Glomus* in isolated species has also been reported for other metal-contaminated ecosystems, such as a Cu mining area of north eastern Brazil (*da Silva et al. 2005*) and a chemical contaminated site of Northern Italy (*Vallino et al. 2006*). The abundance of AM fungal spores ranged from 16 to 210 spores per 10 gm of soil. Variations in AM fungal spore frequency possibly implied that differences could exist in their tolerance to heavy metals. A higher tolerance to Zn, Cd and Pb of indigenous AM fungi from polluted soils in comparison to those of reference isolates from unpolluted soils has been reported (*Weissenhorn et al. 1993; Diaz et al. 1996*).

*Pawlowska (2000)* also found that *G. constrictum*, *G. mosseae* and *Glomus* sp. were dominant species in Cd contaminated soils. These five species identified (*G. macrocarpum, G. mosseae, G. fasciculatum, G. fugianum* and *G. constrictum*) should be isolated and tested in relation to their potential use as an inoculum in phytoremediation programs in these areas.

The present study indicated that the first and second highest abundance of spores were recorded in the rhizosphere soil of Garden and Yamuna soil mainly for *Glomus.sp* with 210 spores per 10 gm of soil, respectively. Secondly, it is known that heavy metals can delay, reduce and even eliminate spore germination and AM colonization. Increasing Zn and Cd could reduce AM fungal spore germination and hyphal growth (*Weissenhorn*...
et al. 1994) and subsequently reduce or even eliminate AM infection (Koomen et al. 1990). Del Val et al. (1999) showed that the total number of AM fungal spores correlated negatively with the metal content of the soil.

Similarly, Daiz and Honrubia (1993) used Medicago sativa to show that mine spoils and waste sediments containing Zn and Pb had mycorrhizal potential, although the numbers of AM fungal spores were lower than the adjacent soil not altered by mining activity.

Small variation in spore density of AM fungi recorded from metal-contaminated site in the present study is contradictory to the findings of Zak et al. (1982) who reported 390–2,070 spores/100 g substratum in mine spoils of Canada. Furthermore, independent studies examined spore count and colonization efficiency of sewage sludge-treated sites and revealed that spores tolerant to increased HM application readily colonize host roots despite low spore counts (Del Val et al. 1999; Jacquot-Plumey et al. 2001), and therefore, higher colonization rates reported in the present study could be due to favorable time for spore germination and rapid colonization of emerging new roots of the plants resulting into low-spore density in rhizosphere soil. Our study reported low species diversity of arbuscular mycorrhizal fungi in rhizosphere of plants sampled from metal-contaminated than non contaminated soils, and this result can be attributed to low spore density of AM fungi in rhizosphere soils. The cadmium-rich soils in the present study harboured less species of AM fungi in rhizosphere soil of naturally growing plants. Similarly, single species of arbuscular mycorrhizal fungi was recorded by several authors in Zn-, Cu-, Pb-, Ni-, Cd-, and Mn-
enriched soils as summarized by Griffioen (1994). Diverse AM fungi (Glomus and Gigaspora species) have been reported by Raman et al. (1993) in the mycorrhizosphere of 14 plant species colonizing a magnesite mine spoil in India. Similarly, Gonzalez-Chavez et al. (2002) reported the diverse arbuscular mycorrhizal fungi from arsenic mine spoil with representatives from the genera Glomus (Glomus caledonium, Glomus claroideum, Glomus constrictum, Glomus fasciculatum, Glomus intraradices, and two unidentified Glomus species), Acaulospora (Acaulospora delicate and Acaulospora undulata), and Enterophospora (Enterophospora infrequens).

In the present study, Glomus species were predominant in the rhizosphere of the plants growing on metal contaminated site and non contaminated site and in the case of non contaminated site, Glomus sp. was common to all plants and all plants were modestly colonized (Vallino et al. 2006).

As the concentration of Ni and Cd has been already reported from treated water (http://www.usembassy.gov), in the present study, we undertook rhizosphere soil for determining the concentration of Cd and other metals in the soil where they form stable complexes. In contaminated soil, profuse hyphal colonization and intracellular arbuscular colonization, the functional stage of AM symbiosis along with intercellular vesicular colonization indicated Arum type of arbuscular mycorrhiza (Vyas et al. 2003), while in non contaminated soil, the presence of intercellular hyphae growing through longitudinal intercellular air spaces, intracellular hyphal coils, intracellular arbuscules, colonization also indicated Arum type of mycorrhiza (Vyas et al. 2003). The longitudinal spread of hyphae occurs because hyphae grow
through longitudinal intercellular air spaces (*Brundrett et al. 1985*), and a relatively rapid parallel spread of intercellular hyphae occurs along these channels. The resulting colonies of arbuscular mycorrhizal fungi have a linear appearance. Further, the presence of globose and ellipsoidal vesicles, represented AM fungi belonging to genus Glomus in contaminated and noncontaminated soil. Thus, different stages of root colonization by arbuscular mycorrhizal fungi recorded in the present study may be attributed to time of sampling (monsoons). Also, arbuscular mycorrhizal fungi propagate by spores, hyphae, and colonized root fragment, and the relative importance of these different propagules varies depending on the environmental conditions (*Smith and Read 1997*). Similarly, mycorrhizal colonization (hyphae, arbuscules, and vesicles) in soils contaminated with tannery sludge and effluents may be more adversely affected by Cd. However, high colonization still occurred at most toxic Cd concentrations. The present study reported arbuscular colonization which plays an important role in mineral ion exchange in root cortical cells and shows greatest sensitivity to high chromium levels, and its occurrence reveals AM enhanced plant accumulation and tolerance to Cd (*Davies et al. 2001*).

In the present study, for a particular plant species, root colonization, spore density was higher in non-contaminated soils compared to contaminated soils. This may be due to higher electrical conductivity in contaminated soils resulting in greater availability of soluble metals, leading to increase AM fungal mediated uptake by plants (*Raju et al. 1990; Liao et al. 2003*), and the effect of heavy metals on mycorrhizal occurrence and infectivity seems to be more of a function of the available rather than total content in the soil.
Further, the high colonization of AM fungi in plants from non-contaminated soils may also be aiding the plants to cope up against elevated concentrations of nutrients, too, resulting in increased growth (Bi et al. 2003) and successful survival of the species (Bi et al. 2003) under salinity stress caused by high metal concentrations elevating its negative effect on the plants (Dueck et al. 1986), thereby imparting resistances (Gildon and Tinker 1981; Weissenhorn et al. 1994a, b and Weissenhorn and Leyval 1995). Further, our findings are in accordance with Turnau et al. (1996) who reported that metal tolerant Oxalis acetosella plants colonizing acid forest soils treated with Cd–Zn and Pb containing industrial dust showed even higher colonization than non-treated soils.

Furthermore, independent studies examined spore count and colonization efficiency of sewage sludge-treated sites and revealed that spores tolerant to increased HM application readily colonize host roots despite low spore counts (Del Val et al. 1999; Jacquot-Plumey et al. 2001), and therefore, higher colonization rates reported in the present study could be due to favorable time for spore germination and rapid colonization of emerging new roots of the plants resulting into low-spore density in rhizosphere soil. Our study reported low species diversity of arbuscular mycorrhizal fungi in rhizosphere of plants sampled from metal-contaminated than non-contaminated soils, and this result can be attributed to low spore density of AM fungi in rhizosphere soils.

Further the results indicate a definite correlation between level of mycorrhizal infection in the roots and the spore counts of the soil s around
them. The more is the number of spores in the rhizosphere soil, the more is the degree of colonization. The present study is in accordance with the above studies.

Vetiver is native to India, its environmental application for soil and water conservation is traditionally practiced for longer time. Systematic efforts to develop the vetiver grass technology for mitigation of soil erosion and water conservation were first initiated in India; however it was not practiced seriously. Several countries on the other hand, taking cues from the Indian initiative extensively implemented environmental applications of this grass (Lavania, 2004). Currently two main methods for treating contaminated water namely, ‘engineering’ and ‘biological’ are being used. The biological method consists of land irrigation, wetland and hydroponics system (Chomchalow, 2003).

As vetiver has been found to be highly tolerant to extreme soil condition including heavy metal contamination, the present study was conducted to check the reduction level of Ni contaminants of soil on the plant’s ability to tolerate toxic levels of Ni and on the ability to accumulate these heavy metals in roots and shoots. Truong and Baker (1988) have proved the similar results. It might be concluded that heavy metals in soil even at the higher level to plant growth have no negative effect on vetiver root growth but showed negative effect on the shoots and the growth of shoots sustained after some days so as the growth of plant. Our study did not in coordination with the mentioned results. (Roongtanakiat and Chairoj, 2001).
For successful phytoremediation, the first requisite is high production of plant biomass. Since metal removal is a function of metal concentration in the harvestable biomass, the plant should be able to produce enormous biomass so that it can grow successfully at the contaminated site. Cr uptake is an important parameter in understanding the cellular responses of high HM concentration in plants and is one of the requisites contributing to the success of phytoremediation. The ambient metal concentration in the soil was the major factor influencing the metal-uptake efficiency as the metal uptake was observed to increase with increase in treatment doses (Ghosh & Rhyne 1999; Begonia et al. 2005). Considering the definition of hyperaccumulator given by Foy (1984), Baker & Brooks (1989), Baker et al. (2000) and Reeves & Baker (2000), Indian mustard behaves as a potential Cr hyperaccumulator. The present study is not in accordance with the above mentioned results because plant chosen in the present study has a higher biomass of roots as compared to arial parts. Pods are also used commercially for essential oil extraction (Khus-Khus) which is used in perfumery and beverage industry. Also the roots of the plant are extensively used for making Chiks (a type of curtain sprayed with water occasionally in summer months and keep the area cool and gives sweet fragrance at the same time. Due to higher biomass of its roots the plant is also used for keeping a check on soil erosion.
SECTION 7
CONCLUSIONS

On the basis of the above results following conclusions could be drawn

1. The spore count decreases with the onset of colonization and the increases with when root percent colonization reaches at least 70%.

2. The inoculum produced from the spores extracted from contaminated soil showed better percent colonization as well as better tolerance index of the plants symbiotic with them.

3. Root biomass and shoot biomass was better in inoculated plants as compared to non-inoculated plants.

4. In 1st year and 2nd year of growth the plants in contaminated soil showed deficiency symptoms and their tolerance index was also lower then control plants but in 3rd year the inoculated plants growing in contaminated soil showed better tolerance index of more them 1 as compared to control.

5. The above conclusion showed that the Vetiver can survive well in contaminated soil if AM fungal inoculum is used. In other words symbiosis with AM fungal spores of more than 70% helped plant to thrive well in polluted soils.