CHAPTER - I

INTRODUCTION
1.1 Importance of fertilizers in soil fertility, growth of plants and crop yield.

No plants, no life. This fact is true in all its narrow and wide senses. Even though we are not aware of this truth, plants play the key role in maintaining life on earth. Today plants have been exploited for myriads of human needs such as food, fodder, shelter, medicine, etc. and hence to replenish them in a proper way should be our prime concern. To cultivate plants by using eco-friendly cultivation practices is one of the important ways to replenish the plants that have been exploiting for human needs.

The growth and yield of plants are largely determined by the fertility of the soil. The soil fertility is primarily dependent on the quality and quantity of nutrients present in the soil as well as their availability to plants. The availability of nutrients to plants is controlled by many soil parameters like soil aeration, soil texture, and soil size, type of soil, water content and water holding capacity of soil, porosity of soil and so on. To some extent, most of these parameters are intimately connected with the organic and inorganic substances in the soil, which are the soil nutrients. Soil is the source of 13 of the 16 essential plant nutrients. The concentration of these nutrients and the condition making them available to plants are of fundamental importance to plant growth (Miller and Donahue, 1992). These 13 nutrients are available to plant only from the soil by absorption through roots and hence they are called mineral elements. Among these 13 elements nitrogen, phosphorus, potassium, sulphur, calcium and magnesium are needed in large quantities, which are named as macro nutrients or major elements. Whereas, copper, boron, chlorine, iron, manganese, molybdenum and zinc are considered as micro nutrients or minor or trace elements as they are needed in trace quantities. Some soil scientists included iron under the category of major elements, as it becomes a limiting factor in fewer quantities for many crop plants (Noggle and Fritz, 2004).

Continuous cultivation of crop plants depletes the nutrient content of soil and hence, to support plant growth, these nutrients are to be supplied from outside as fertilizers. The addition of fertilizers can be in the form of inorganic, organic or
biofertilizers individually or in different combinations. Each nutrient has a special role in the metabolism of plants. The deficiency of each would block the metabolism and thus negatively affect the growth and yield of plants. Hence, the fertilizers are added to the soil after analyzing the nutrient level of the soil.

1.1.1 Inorganic fertilizers

These are chemical compounds synthesized artificially in factories. Here the chemicals are present in high doses in pure form, which rapidly change the quality of the soil (Miller and Donahue, 1992). Though the chemical fertilizers are known to enhance the NPK level of the soil and support plant growth, continuous application of these chemicals reported to affect the soil fertility in the long run (Babidha et al., 2010). Continuous use of inorganic fertilizers leads to undesirable changes in the soil and environment, leading to lose of biological dynamics and fertility of soil, ultimately endangering the sustainability of the farming.

1.1.2 Organic manures

Organic manures constitute the dead and decayed parts of plants and animals as well as the excreta of animals including vertebrates and invertebrates. Any organic matter can become an organic fertilizer when it is decayed by the microorganisms. Organic manures increase the growth and yield of plants, at the same time improve the physical, chemical and biological properties of the soil (Albiach et al., 2000). The use of organic fertilizers is a promotive practice in the present day agriculture and horticulture. The very commonly used organic manures are farmyard manure, green manure, cattle dung, poultry manure, vermicompost, etc.

The green manure is known to improve soil structure and decrease leaching loss of nutrients. The decomposition of organic matter in the green manure resulting in the liberation of CO₂ influences the weathering of minerals and release of nutrients (Singh et al., 1982). It also helps to accumulate biomass, which in turn favourably affect the soil texture. Farmyard manure plays an important role in increasing soil fertility and texture. It is reported to act directly to increase the crop yield by accelerating the respiratory process and cell permeability of the plants or
by hormonal action (Gaur, 1991). Indirectly it improves the physical properties of soil such as aggregation, aeration, permeability and water holding capacity. Poultry manure is reported to contain higher quantities of nitrogen and phosphorus and potassium (Malone et al., 1992). Cow dung and sheep dung are readily available and cheap organic manures, which increase the soil properties and thereby increase the crop yield. The composted cattle manures are highly valuable due to the presence of decomposed organic matter and innumerable microorganisms which increase the micro flora of the soil.

Vermicompost is a mixture of worm casts, which is a rich source of micro and macronutrients. Vermicomposting is a process of recycling of organic wastes in an environmentally safe method. It increases the density of microbes and provides the required nutrients to the plants. It contains plant growth promoting substances such as, NAA, cytokinins, gibberellins, etc. and N, P₂O₅, K₂O and Fe (Girradi, 2001 and Girradi et al., 2006). Vermicompost being a stable fine granular organic matter, when added to clay soil loosens the soil and improves the passage of air. The mucous associated with the worm cast being hydroscopic, absorbs water, prevents water logging and improves the water holding capacity. The organic carbon in vermicompost releases the nutrients slowly and steadily into the system and enables the plants to absorb these nutrients. The soil enriched with vermicompost provides additional substances that are not found in chemical fertilizers (Kale et al., 1992).

1.1.3 Biological fertilizers or Biofertilizers

As soil microorganisms are active transformation agents of both the mineral and organic components of the soil, they play a key role in the plant nutrient cycle, especially in soil fertility. They enhance the soil fertility by acting on the organic materials in the soil and releasing the nutrients in the available form, by producing and relieving enzymes into the soil, by fixing the unavailable form of nitrogen into available nitrogen compounds, etc. Use of biofertilizers proved as a remedial substitute for chemical fertilizers in providing nutrients to crops and maintaining soil health and thus the sustainability of the soil (Bhardwaj and Gaur, 1970).
Among the different microbes, especially bacteria experimented, Azospirillum was found to be better in the tropical and temperate conditions. It is reported that Azospirillum occurs in about 90% of tropical soil and in nearly 60% of soils of temperate climate (Reynders and Vlassak, 1982). The effect of inoculation of bacteria to a large extent, dependent on their ability to survive in the soil.

The free living soil bacteria, Azatobactor and Azospirillum are widely employed for cultivation of several plants like Cabbage (Jeevajyothy et al., 1993), garlic (Wagne, 1996), pepper (Kanthaswamy et al., 1996), snake gourd (Elizabeth et al., 2000). Sebastian & Mathew (2006) reported that the soil nitrogen fixing bacteria, Azospirillum added to the soil increased the fixation and utilization of nitrogen, promoting better growth and yield in tissue culture banana. So far no reports are there regarding the effect of Azospirillum alone or in combination with NPK and/or vermicompost on growth and yield of *Moringa oleifera* PKM-1 variety. Beaulah et al. (2004) studied the canopy spread of *M. oleifera* PKM-1 by the addition of Azospirillum with organic fertilizers such as FYM, neem cake, poultry manure, panchagavya, etc. and significant increase in canopy spread was reported. Hence, in the present study an attempt was made to study the effect these fertilizers, alone or in various combinations on the growth and yield of *M. oleifera* PKM-1 variety. Further, the type of fertilizer used may change the quantity and quality of biochemical contents in the Moringa seed. It is to be noted that the biochemical compounds contained in the various parts of Moringa plant is responsible for the water purification.

1.2 Importance of water and the problems of water pollution

Water is the most essential part of all living systems and is the medium in which life exists. Even though 70% of the earth’s surface is covered with water, only a relatively small percentage is actually involved with terrestrial, atmospheric and biological processes (Manahan, 2010). Human welfare is determined by many factors, of which the quality and quantity of drinking water is one of the major concern. More than six million children in the world die every year through intestinal infection caused by drinking contaminated water (Litherland, 1995). In
the developing countries drinking water problem is as severe a problem like food problem, especially in the rural areas.

The waste water releasing from different industries and factories is a major cause of water pollution. The wider and intensive use of artificial fertilizers indirectly increases the growth of microorganisms in the water bodies, as they leach out from the soil and raise the nutrient status of water reservoirs, resulting in eutrophication. Rain water washes out animal wastes and domestic sewage from the shores of rivers and ponds. Most of our water sources are used as laundries polluting the water to a great extent. The most important cause for manmade microbiological contamination of the water is water use habits, dependent on socio-cultural factors like disposal of domestic sewage from the human habitations, faecal contamination from human and animal wastes, etc.

Another important cause of water pollution is the toxic metals released from many industries. The heavy metals are of special interest, because they are non-degradable and are persistent even after long years, if the industrial waste water has not undergone proper treatment. The common heavy metals which are found to be present in industrial waste waters are lead, copper, chromium, arsenic, zinc, cobalt, nickel, etc. These are toxic in elemental as well as compound forms. Exposure of these metals, even in very minute concentrations is proved to be harmful to human health (Sekharan et al., 1995; Selvarani, 2000; Arivoli, 2007).

The polluted waste water is usually found to have very high or very low pH, high turbidity, electric conductivity (EC) and total dissolved solids (TDS). High BOD and COD is a characteristic feature of polluted waters, which in turn decrease the content of dissolved oxygen (DO). Salinity also may be increased according to the type of salts dissolved in water. Presence of calcium and magnesium ions and other positive inorganic ions in the waste water leads to the hardness of water. Other commonly present ions are chloride, fluoride, ammonium, nitrite, nitrate, phosphate, sulphate, etc. High concentrations of inorganic and organic pollutants lead to the high frequency growth of microbiological flora and fauna which will reduce the DO and enhance the BOD.
1.2.1 Methods of treatment of polluted waters

A very common process of purification of water is the use of different types of coagulants, which flocculate the polluting particles together and sediment down or the flocculate is filtered off to get the clear water. The effluents from industries pour out into the environment after treating with this type of coagulants. Many chemical substances, mainly metal coagulants such as aluminium sulphate, sodium aluminate, potash alum, ammonium alum (aluminium based coagulants), ferric sulphate, ferrous sulphate, chlorinated ferrous sulphate and ferric chloride (iron based coagulants) are commercially available and are very commonly used for the treatment of municipal water supplies (Hammer and Hammer, 2008), in the water treatment plants. These high technology solutions provide drinking water to multitudes of people and brought great progress to the social development of many remote areas during the past years. However, most of the physical and chemical processes used to treat water are not safe to depend, as the treated water may be hazardous to human health.

The polyaluminium chloride and alum, used for the treatment of polluted water add impurities such as epichloridine to water. Such impurities are reported to be carcinogenic (Muyibi and Alfugara, 2003, Ghebremichael et al., 2005). Aluminium is regarded as an important poisoning factor in dialysis encephalopathy. Aluminium also is one of the factors which might contribute to Alzheimer’s disease. Alum reacts with water alkalinity reduces water pH and its efficiency in cold water (Katayon et al., 2006). Above all developing countries pay a high cost to import chemicals including polyaluminium chloride and alum, making the chemical treatment of polluted water costly and unaffordable (Ghebremichael, 2004). Hence, there is a strong urge among the nations, to search for low cost methods, using natural materials.

There are many traditional methods for the improvement of rural water supplies in the tropics among the village people, by using available raw materials. But many of these methods found to be harmful to health and do not please the senses. In attempts to make scientific assessment of the different types of
traditional methods, Jahn (1981) identified domestic water coagulation as one of the most promising ways to obtain clear and appetizing water and simultaneously remove high percentage of microbes.

1.2.2 Use of natural coagulants in the purification of polluted water

Different materials of biological origin had been tested for purification of water by coagulation, sedimentation and /or filtration. Strychnos seeds rubbed in little water and added to polluted water have the capacity to coagulate and sediment all the suspended impurities from muddy water as a precipitate (George, 1893). In the same way seeds of *hypoestes verticillaris, Blepharis persica, vicia faba, Moringa oleifera, M. Stenopetala* and *Trigonella* sps. and bark of *Boscia senegalensis* were found to be able to coagulate and sediment the impurities of polluted water (Jahn, 1986). Among which seeds of *M. oleifera* was observed to be most efficient and readily available material (Amagloh and Benang, 2009).

Arab women in Sudan had discovered that muddy water of the Blue Nile could be clarified within 1 to 2 hours by swirling 150-200 mg of cloth wrapped Moringa seed powder in a container (Barth *et al.*, 1982). The majority of traditional flocculating materials seemed to be much weaker than alum, but locally available *M. oleifera* seeds were found to act as primary coagulants and as effective as alum when properly used (Jahn and Dirar, 1979; Jahn, 1981). Ademiluyi and Eze (1990) reported that *M. oleifera* seed marc (oil free seed power) is a cheaper promising water clarifying material. Sani (1990), Folkard and Sutherland (2001), Amagloh and Benang (2009) observed the removal of high percentage of turbidity, hardness, BOD, COD, alkalinity, etc. from polluted waters by using Moringa seed powder. Studies of Sajidu *et al.* (2005) and Sharma *et al.* (2006) showed that *M. oleifera* seed powder is effective in heavy metal remediation of water. The scientists at the institute of toxicology and chemotherapy at Heidelberg, Germany after their investigation reported that the use Moringa seeds as water purifier may not constitute any health hazards (Berger *et al.*, 1984).
1.3 Antibacterial activity of plants and plant products

According to World Health Organization (Anon., 2008) more than 80% of the world’s population relies on traditional medicine for their primary health care needs. The reason may be that the plants and plant products are experienced to have important curative properties and are safe and efficient to use. One property related to the medicinal importance of plants is the antimicrobial effect which attributes great significance in therapeutic treatments. Several workers throughout the world have carried out antibacterial studies on large number of plants. It has been shown in certain studies that plants protect themselves against microbial pathogens by various defense responses including production of antimicrobial peptides, secondary metabolites, lytic enzymes and membrane interacting proteins (Feng et al., 2003). Such plants or plant products also may have water purifying effects on treating with polluted waters, since microbes, especially the bacteria are a major constituent of the polluted water.

Previous studies have reported many antimicrobial peptides isolated from common vegetables and spices (Ngai and Ng, 2004; Talas, 2004; Mariangela et al., 2006). Rahman and Khanum (2011) isolated and characterized the antimicrobial peptides from the crude extracts of *Pisum sativum* L. and their antibacterial activity was tested against a number of bacteria using disc diffusion method.

Ghebremichael et al. (2005) reported that coagulant proteins showed both flocculating and antimicrobial effects. Hence, the coagulation of pollutants is related to the antibacterial property of the organic material used for the treatment of the polluted water. *M. oleifera*, wild variety seed powder is reported to have antibacterial activity (Buker et al., 2010; Vieira et al., 2010; Futi et al., 2011; Nwaiwu and Bello, 2012) demonstrating inhibition of growth of the bacteria. There are studies regarding the presence of antimicrobial peptide and membrane interacting proteins, characterized by a net positive charge and amphipathic nature which allows them to persist at water-lipid interface and then disturb the microbial membrane components (Ruissen et al., 2001 and Feng et al., 2003). But so far no reports are there regarding the antibacterial effect of the seed powder of PKM-1
variety. Hence an evaluation of the antibacterial effect of the seed powder of PKM-1 variety was done in this study using the seed powder extracts in various solvents by screening against certain pathogenic bacteria.

### 1.4 In vitro culture of plants for antibacterial studies

*In vitro* culture is used as the resources of secondary metabolites, mainly because they can be isolated rapidly and efficiently in comparison to extraction from complex whole plants. Other advantages of the production of antibacterial products by *in vitro* culture are that, the production can be more reliable, simpler and predictable. The compounds produced *in vitro* can be directly parallel to compounds in the whole plant. Another factor is that the interfering compounds that can occur in the field grown plant can be avoided in the cultures and that can be a source of defined standard phytochemical in large volumes. Secondary products by tissue culture can be generated on continuous year-round basis without any seasonal constraints.

In many cases the antibacterial activity of the callus can be compared with that of the original plant material. But there are reports having reduced or no antibacterial activity for the *in vitro* plant extracts, even when the natural plant parts showing very effective antimicrobial activity (Jana and Shekhawat, 2010). The reason is that even though in many cases the callus cultures are a good source of all the secondary metabolites produced in the mother plant, in certain cases one or few phytochemicals are produced in the *in vitro* culture (Facchini and Bird, 1998). Also the intensity of the phytochemical is quite low in the callus extracts in comparison to the direct plant extract (Salvadore *et al.*, 2003).

Studies on the antibacterial effect of callus from Moringa explants are not reported. Katherine *et al.* (2004) reported that although many of the thirteen known Moringa species are in danger of extinction, one species, *M. oleifera* Lam., is now widely cultivated. *M. oleifera* PKM-1 variety was therefore utilized to develop callus and extract secondary metabolites from it. Islam *et al.* (2005) reported the propagation of *M. oleifera* plants from nodal explants.
1.5 Phytochemical analysis of plant products showing antibacterial and coagulating activity

Higher plants are a source of multitude of phytochemicals as carbohydrates, proteins, fats and secondary metabolites. They may be active in various situations or unique in their activity in special conditions. Certain antimicrobial peptides and membrane interacting proteins are reported from certain vegetable plants and spices having antibacterial effect (Ngai and Ng, 2004; Hu et al., 2004; Oard et al., 2004; Talas, 2004; Mariangela et al., 2006). Many plant seeds such as Abelmoschus esculentus, Strychnos potatorum, Prosopis juliflora etc. were tested and observed to have the coagulation activity and reported that some cationic or anionic polymers are the active agents of coagulation (Yin, 2010). According to Bolto and Gregory (2007), polymeric coagulants can be cationic, anionic or non-ionic, in which the former two are collectively termed as polyelectrolytes. The water soluble kernel crude protein of wild Moringa was reported as 36.8% by Foidl et al. (2001), which is responsible for the coagulation of pollutants in water. Yin (2010) stated that natural coagulants are mostly either polysaccharides or proteins. He also suggested that the existence of background electrolytes in aqueous medium can facilitate the coagulating effect of polymeric coagulants since there is lesser electrostatic repulsion between particles. Plant proteins were at one time specifically, classified on the basis of solubility properties (Osborne, 1924) and this is still a useful criterion for distinguishing different types of storage proteins (Harborne, 1973). The classes are separated according to their solubility or lack of solubility in water, salt solution, aqueous acid and alkali and 70% alcohol.

1.6 Taxonomy and Morphology of Moringa oleifera Lam.

Moringa oleifera comes under the family Moringaceae. This family embraces only one genus Moringa, which was in the past, credited with only three species. Today, the number is 10 to 12. The best known and most widely distributed species is Moringa oleifera Lam. The synonyms are Moringa pterygosperma Graertn, Moringa moringa (L) Millsp, Moringa nux-ben per,
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Hyperanthera moringa wild, Guilandina moringa Lam. (Morton, 1991) and Moringa zeylanica (Dalzell and Gibson, 1861).

The plant *M. oleifera* is commonly known as horse radish, radish tree or drumstick and they are small or middle sized trees with soft white wood (Dalzell and Gibson, 1861; Patel, 1943; Peter, 1979 and Ramachandran *et al.*, 1980). Leaves deciduous, alternate, 2-3 pinnate, the pinnae and leaflets are imparipinnate, opposite, glandular at the base, stipules absent, flowers large, irregular, hermaphrodite in axillary panicles. Calyx cup shaped with 5-clefts, the segments unequal, petalloid, imbricate, petals 5, unequal, the upper small, and the lowest largest. There is the presence of disk lining the calyx tube. Stamens inserted on the margins of the disk, declinate, 5, perfect, opposite to the petals with 5 to 7 alternate sterile antherless staminodes, filaments free, thickened at the base; anthers one celled dorsifixed. Ovary stipulate, 1-celled, style slender, tabular, stigma truncate, perforated, ovules many, biseriate on 3 parietal placentas. Fruits elongate, 1-celled, loculicidally 3-valved, beaked capsule corky and pitted within. Seeds many in the pits of the valves, testa corky, winged, cotyledons plano-convex, radicle very short superior, plumule many leaved (Gamble, 1997).

1.6.1 The *oleifera* species is marked by the following characters

It is a small or middle sized tree (Dalzell and Gibson, 1861; Patel, 1943; Peter, 1979; Ramachandran *et al.*, 1980) ranging in height from 5 to 10 or almost 15 meters. It has tuberous roots, corky, whitish bark, soft spongy wood, short trunk and slender or wide spreading or drooping fragile branches (Morton, 1991). The alternate twice of thrice-pinnate leaves are spirally arranged mostly at the branch tips (Dalzell and Gibson, 1861; Ramachandran *et al.*, 1980; Morton, 1991). The flowers pleasantly fragrant are borne profusely in axillary drooping panicles 10-25cm long, white or cream coloured, yellow dotted at the base (Md. Salarkhan, 1973; Morton, 1991). The curious fruits borne singly or in pairs are light green, very slim, tender and dangling at first, gradually thicken to 2 cm wide, 25-45 cm long, dark green and firm and finally become brown and thinly woody. When fully mature split open into three valves and a single row of triangular, brown to black,
seeds each with three white papery wings embedded in dry, white tissues like pith (Benthall, 1946; Corner, 1952; stone, 1970; Martin and Ruberte, 1975 and Mabberley, 1987).

1.6.2 Production of *M. oleifera* PKM-1 variety

Scientists at the Horticultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Periakulam of South India by judicious breeding programmes including introduction of elite mother plants, evaluation, selection and hybridization developed the seed propagated Moringa type PKM-I. (Sathashakthi, 1997). The following characteristics make the PKM–I variety unique.

This variety was evolved through pure line selection. It is a seed propagated, medium or dwarf statured tree, growing up to 2-3 m height. Pods are 60-70 cm long with 6.3 cm girth weighing 120 g. It bears 220-250 fruits per tree. The estimated yield is 50-54 tonnes per hectare. It is suitable for ratoon crop. It has low incidence of insect pest and diseases. This variety is suitable for varied soil types (freely drained) in tropical plains (Rajangam *et al.*, 2001).

1.7 Objectives of the study

i) A systematic evaluation of the effect of organic/ inorganic/ biological fertilizers on growth and yield of PKM-1 variety of *Moringa oleifera*.

ii) To find out the effect of foliar spray of growth regulators in flowering and fruiting of *M. oleifera* PKM-1 variety.

iii) To study the water clarifying effect of the seed powder extracts of PKM-1 (standard and fertilizer treated) and wild variety of *M. oleifera* in various solvents like distilled water, 1% NaCl, 0.1N NaOH, and ethanol.

iv) To study the antibacterial property of *M. oleifera* seed powder.

v) To identify and study the biochemical components responsible for the water purifying ability of *M. oleifera*. 