CHAPTER 2

LITERATURE REVIEW

2.1 Antioxidants

Antioxidants are molecules capable of slowing or preventing the oxidation of other molecules. Oxidation reactions can produce free radicals that are called reactive oxygen species (ROS) and can start chain reactions that damage the cells. The damage caused by free radicals is known as oxidative stress, which plays a role in the development of many diseases including Alzheimer's disease, cancer, eye disease, heart disease, Parkinson's disease and rheumatoid arthritis.

Antioxidants are nutrients (vitamins and minerals) as well as enzymes (proteins in the body that assist in chemical reactions). There are several micronutrients that the body cannot manufacture and these must be supplied in the diet. Moleclues with antioxidant activity include beta-carotene, lutein, lycopene, selenium, vitamin A, vitamin E and vitamin C. These antioxidants are abundant in vegetables and fruits and are also found in grain cereals, teas, legumes, and nuts. Phytochemicals and antioxidant constituents in plant material have raised interest among scientists, food manufacturers, producers, and consumers for their roles in the maintenance of human health (Milner, 1999). Phytochemicals are bioactive substances of plants that have been associated iwith the protection of human health against chronic degenerative diseases. Antioxidants are compounds that help delay and inhibit lipid oxidation (Lako *et al*., 2007). Szeto *et al.* (2001) reported that a single cup of green tea would supply as much antioxidant power as 150 mg of pure vitamin C. One hundred grams of strawberries contain almost the same antioxidant power as 140 mg of vitamin C, which is the same vitamin C content found in 1 kg of Chinese pears. One hundred grams of Chinese kale were found to have as much antioxidant power as 90 mg of pure vitamin C, which is also true for 1.3 kg of iceberg lettuce. Yutthana (2009) found that green leafy vegetables contained antioxidants derived from vitamins A, C, E, or beta-carotene that are beneficial for human health. A study of the antioxidant activities of 22 common vegetables, green tea, and black tea indicated that kale had the highest antioxidant activity against hydroxyl radicals followed by Brussels sprouts, alfalfa sprouts, beets, spinach, and broccoli (Guohua *et.al*., 1996).

Polyphenols are a group of secondary plant metabolites with beneficial health effects. Flavonoids are a group of polyphenols that occur widely in fruits and vegetables. Soltoft *et al*. (2010) concluded that organically grown onions, carrots, and potatoes generally have higher contents of health-promoting secondary metabolites in comparison with the conventionally cultivated ones. Nattakaan *et al.* (2006) studied phenolic compounds and antioxidant activity in fermented juices from tomato, guava, and carrot, which were mixed into four different formulas. Analysis of the total antioxidant capacity indicated that the fermented juice mixture of guava and tomato showed the highest antioxidant activity and contained high levels of ascorbic acid and phenol compounds. Kequan and Liangli (2006) studied the total phenolic contents

(TPC) and antioxidant properties of various vegetables. The results from this study suggested that kale, spanich, broccoli, and rhubarb are good dietary sources of natural antioxidant activities and phenolic compounds.

2.2 Vitamin C

Vitamin C, also known as ascorbic acid ($C_6H_8O_6$) (Figure 2.1), is an important water-soluble antioxidant in biological fluids that protects other substrates from oxidative damage and in reduction reactions it regenerates other antioxidants, such as α-tocopherol, glutathione, and beta-carotene (Carr and Frei, 2002).

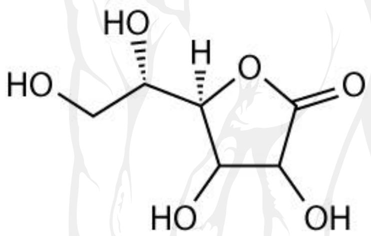
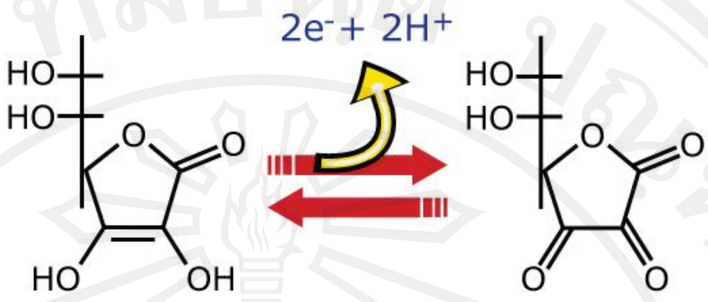


Figure 2.1 The structure of vitamin C (L-Ascorbic acid)

Vitamin C is an important primary metabolite of plants that functions as an antioxidant, and it scavenges superoxide and hydroxyl radicals as well as acting as a chain-breaking antioxidant in lipid peroxidation (Gayosso-García Sancho *et al*., 2011; Wolucka *et al*., 2005). Vitamin C is found in nature in two types, L-Ascorbic acid and L-Dehydroascorbic acid (Figure 2.2).



Ascorbic acid **Dehydroascorbic acid**

Figure 2.2 The oxidation of ascorbic acid

Vitamin C is essential for the formation, growth, and repair of bone, skin, and connective tissue. Connective tissue is especially important because it binds other tissues and organs together and includes tendons, ligaments, and blood vessels. Vitamin C helps maintain healthy teeth and gums. It helps the body absorb iron, which is needed to make red blood cells. Vitamin C also promotes the healing of burns and wounds and has a wide variety of uses in the body. Vitamin C has received special attention as a promising chemopreventive agent for cancer. It helps to slow down or prevent cell damage and it is needed to maintain healthy body tissues and the immune system. Vitamin C is found abundantly in many fruits and vegetables such as citrus, tomatoes, cabbage, broccoli, Brussels sprouts, bean sprouts, cauliflower, kale, mustard greens, red and green peppers, peas, and potatoes. It is commonly recognized as a naturally occurring nutrient and an important antioxidant in the diet.

After reviewing 41 published studies and comparing the nutritional value of organically-grown and conventionally-grown fruits, vegetables, and grains, Worthington (2001) concluded that organic crops contained significantly more of several nutrients. These findings included: 27.0% more vitamin C, 21.1% more iron, 29.3% more magnesium, and 13.6% more phosphorus. In addition, the organic products had 15.1% less nitrates than their conventionally-grown counterparts.

2.3 Plant growth promoting microorganisms (PGPM)

Many studies have shown that crop yield and nutritional quality were improved by organic fertilizers and/or beneficial microorganisms. Organic fertilizers promote the growth and activities of microorganisms and vice versa the value of organic fertilizer (humus) is created mainly by microorganisms. For these reasons, the major factors enhancing yield and nutritional quality are the effect and interaction of both humus (humic substances) and beneficial microorganisms. Elazar *et al*. (1989) found that *Azospirillum* could increase the root surface area of maize. Plants inoculated with *Azospirillum* increased the amounts of both free and bound indole-3-acetic acid (IAA) in the roots. Ravi *et al*. (2004) concluded that inoculation of *Azospirillum* strain OAD-2 significantly increased plant height, number of leaves per plant, branches per plant, and total dry mass accumulation in *Gaillardia pulchella* compared to other inoculations and/or uninoculated control. The nitrogen uptake of *G. pulchella* was also enhanced due to *Azospirillum* strain OAD-2 inoculation. The study by Ordookhani *et al.* (2010) indicated that phytochemical factors containing lycopene and antioxidant activity as well as shoot and fruit potassium were increased by PGPM (*Pseudomonas putida*, *Azotobacter chroococcum*, and *Azospirillum lipoferum*) and AMF (*Glomus intaradics* + *Glomus mossea* + *Glomus etunicatum*) compared to uninoculated control. Maximum lycopene and antioxidant activity were found in plants treated with PGPM + AMF.

PGPM are beneficial bacteria, fungi, and actinomycetes that colonize plant roots and enhance plant growth by a wide variety of mechanisms such as nitrogen fixation, IAA production, and phosphate solubilization. Madhaiyan *et al*. (2009) studied co-inoculation of *Azospirillum* sp. with *Methylobacterium* from the roots of various plants from Chungbuk province, South Korea. The inoculation potential of strain *A. brasilense* CW903 on growth and nutrient uptake of rice, red pepper, and tomato was measured and the result was that the inoculation was able to enhance nutrient uptake in the various crops. Gholami *et al*. (2009) studied the inoculation effect of six bacterial strains in maize including *P. putida* strain R-168, *P. fluorescens* strain R-93, *P. fluorescens* DSM 50090, *P. putida* DSM291, *A. lipoferum* DSM 1691, and *A. brasilense* DSM 1690. The study showed that seed inoculation significantly enhanced seed germination, seedling vigor, leaf and shoot dry weight, leaf surface area, plant height, 100 seed weight, and number of seed per ear. Pandiarajan *et al*. (2012) studied three different species of *Azospirillum* strains from various crop soils of Srivlliputtur Taluk. They concluded that the strains of *Azospirillum* helped the plants grow better by more efficiently utilizing various parameters from the soil. These strains are being used as very efficient biofertilizers to grow crop plants all over the world. Kim *et al*. (2010) reported that *A. brasilense* strains CW301 and CW903 showed the highest acetylene reduction activity and ability to colonize wheat root. They also found that these strains enhance crop growth and nutrient uptake in red pepper, tomato, and rice. Inoculation of *A. brasilense* CW903 appreciably enhanced the accumulation of other nutrients in rice and red pepper.

Sudhakar *et al.* (2000) isolated *Beijerinckia indica* from mulberry rhizosphere along with other nitrogen fixing bacteria. Barbosa *et al.* (2003) isolated *Beijerinckia derxii* from tropical soil and found that it releases certain plant growth regulators. *Beijerinckia derxii* increased nitrogenase specific activity during the stationary phase. Halsall (1993) showed that the nitrogenase activity of *Beijerinckia indica* B15 was stimulated by co-inoculation with *Cyathus stercoreus* both in axenic culture and in native soil.

Hemant *et al*. (2011) studied six actinomycetes isolates, namely *Streptomyces toxytricini* vh6, *Streptomyces flavotricini* vh8, *Streptomyces toxytricini* vh22, *Streptomyces avidinii* vh32, *Streptomyces tricolor* vh85, and vh41, which is an isolate of an unknown species of actinomycetes. The isolates were tested for their efficacy in protecting tomato (*Solanum lycopersicum*) against Rhizoctonia solani under green house conditions. The results showed that all of these actinomycetes have the potential to act as biocontrol agents and promote growth in terms of high chlorophyll content, high phenylalanine ammonia lyase (PAL) activity, and high total phenolic content. Barea *et al*. (2010) studied thirty actinomycetes strains isolated from the rhizosphere of field-grown plants (*Trifolium repens* L.). They found that inoculation of clover plants with either of the selected actinomycetes enhanced plant growth and nitrogen acquisition. Co-inoculation of actinomycetes and *Glomus mosseae* produced synergic benefits on plant growth, MCR9, MCR24, and also on phosphate acquisition.

The three selected actinomycetes improved AM formation by clover plants and *Glomus mosseae*.

2.3.1 Nitrogen fixing ability of beneficial microorganisms

Approximately 80% of the atmosphere consists of nitrogen gas (N_2) but plants cannot use atmospheric nitrogen directly to make protein. Nitrogen (N) is a major nutrient required for plant growth and is taken up by the roots from the soil preferentially as nitrate (NO_3^-) or ammonium (NH_4^+). Thus, gaseous nitrogen must first be converted or "fixed" into the forms NH_4^+ and NO_3^- that plants can use. In fact, under natural conditions there is a slow release of nitrogen into the air as decomposition proceeds but there is also an uptake of nitrogen similar in magnitude from the air. The biological fixation of nitrogen may be small but the release is also small during any short interval. Fortunately, atmospheric nitrogen can be used by a number of free-living microorganisms and by those in symbiotic association with higher plants. Under conditions where combined nitrogen is not applied as fertilizer, the nitrogen-fixing symbiotic and non-symbiotic microorganisms are a major factor in the maintenance of organic matter at some equilibrium level.

Nitrogen fixation by beneficial microorganisms occurs by the reduction of atmospheric nitrogen gas (N_2) to ammonia (NH_3) using nitrogenase enzymes that separate the triple bond of nitrogen gas. Only prokaryotic microorganisms such as bacteria, cyanobacteria, actinomycetes, and Archaea can fix nitrogen gas because they contain nitrogenase enzymes and usually are called "The Nitrogen-fixers or Diazotrophs" (Shutsrirung, 2008).

Nitrogen fixation was the first mechanism proposed to explain improved plant growth following inoculation with *Azospirillum*. This was mainly because of an increase in the number of nitrogen containing compounds and the nitrogenase activity in the inoculated plants. Several years later, however, studies showed that the contribution of N_2 fixation by *Azospirillum* to the plant is minimal and ranged from 5 to 18 % of the total plant increase. Bandara *et al.* (2006) suggested that plant growth could be promoted by endophytic bacteria that increased N_2 fixation and phytohormone synthesis.

2.3.2 Phosphate solubilizing activity by beneficial microorganisms

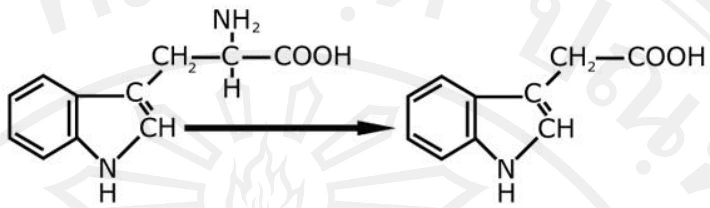
Phosphorus is one of the major essential macronutrients for plants. It is a very important nutrient necessary for plant life because it is needed for plant growth, yield of crops, and required for many metabolic reactions in plants. It functions as one of the major players in the process of photosynthesis, nutrient transport, and energy transfer. Phosphorus also affects the structure of a plant at the cellular level. It is a component of certain enzymes and proteins such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), nicotinamide adenine dinucleotide phosphate (NADP), coenzymes, ribonucleic acids (RNA), deoxyribonucleic acids (DNA), and other materials.

Phosphate solubilizing bacteria (PSB) are a group of beneficial bacteria capable of hydrolyzing organic and inorganic phosphorus from insoluble compounds. The phosphate solubilization ability of microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition. PSB enhance the availability of phosphorus in plants by releasing it from inorganic and organic soil, which has phosphorus pools from solubilization and mineralization. Microorganisms enhance the phosphorus availability to plants by mineralizing organic phosphorus in the soil and by solubilizing precipitated phosphates (Chen *et al.*, 2006). Microorganisms are useful for all of the crops i.e. cereals, cash crops, leguminous crops, horticultural crops, vegetables, etc. Gupta *et al.* (2007) conducted a study on tricalcium phosphate and rock phosphate solubilization with 62 species of fungi and 253 species of bacteria. They found that 12 species of fungi and 19 species of bacteria can dissolve tricalcium phosphate and rock phosphate. Phosphate solubilizing bacteria enhanced the seedling height of *Cicer arietinum* (Sharma *et al.*, 2007), while co-inoculation of PSB and PGPM reduced phosphorus application by 50 % without affecting corn yield (Yazdani *et al.*, 2009). Inoculation with PSB increased sugarcane yield by 12.6 percent (Sundara *et al.*, 2002). Application of PSB with rock phosphate can increase the phosphorus uptake and save about 50% of the crop requirement of phosphatic fertilizer.

2.3.3 Plant growth hormone production by beneficial microorganisms

Plant hormones, called phytohormones, are a group of naturally occurring, organic substances which influence physiological processes at low concentrations. Phytohormones are usually synthesized in one part of the plant and are transported to another location. The processes that are influenced by phytohormones mainly include growth, differentiation, and development, although other processes such as stomatal movement may also be affected. Hormones also determine the formation of flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruit. Plant hormones shape the plant and affect seed growth, time of flowering, the sex of flowers, senescence of leaves, and fruit growth. They also affect which tissues grow upward and which grow downward, leaf formation, stem growth, fruit development and ripening, plant longevity, and even plant death. Hormones are vital to plant growth and without them, plants would be mostly a mass of undifferentiated cells.

Auxins are hormones involved in plant-cell elongation, apical dominance, and rooting. The most common natural form is indole-3-acetic acid (IAA), which was long thought to be derived exclusively from the amino acid tryptophan (Fig. 2.3). Auxins are produced in the apical meristem of the shoot. Developing seeds produce IAA, which stimulates the development of fleshy fruit. For example, the removal of seeds from a strawberry prevents the fruit from enlarging. The application of IAA after removing the seeds causes the fruit to enlarge normally. IAA is produced in actively growing shoot tips and developing fruit. It is also involved in elongation, but before a cell can elongate, the cell wall must become less rigid so that it can expand. IAA triggers an increase in the plasticity, or stretch ability, of cell walls allowing elongation to occur.



Tryptophan **Indole-3-acetic acid**

Figure 2.3 Idole-3-acetic acid (IAA) derived from the amino acid tryptophan

It was found that not only plants but also microorganisms including bacteria, fungi, and actinomycetes are able to produce IAA. *Azospirillum* can produce the phytohormones IAA, gibberellins, and cytokinins *in vitro* (Patten and Glick, 1996; Rademacher, 1994). External application of synthetic hormones or hormones purified from bacterial culture imitated the positive effects of *Azospirillum* on root development and morphology. Tryptophan-dependent IAA production was studied in different *Azospirillum* species and the results revealed that *Azospirillum irakense* KA3 released 10 times less IAA into the medium than *Azospirillum brasilense* Sp7 (Zimmer *et al*,. 1991). Oxygen is required during the conversion of tryptophan to IAA (Bar and Okon, 1995). Soil bacteria use exogenic L-tryptophan for IAA biosynthesis in the root exudates.

Many studies have suggested that the auxin produced by *Azospirillum* is involved in root morphology (Baca *et al.*, 1994; Costacurta and Vanderleyden, 1995). of individual root hairs at least two fold (Dubrovsky *et al.*, 1994). When inoculated onto wheat seedlings *Azospirillum brasilense* SpM7918, a very low IAA producer, showed a reduced ability to promote root system development and decreased the potential of the plants capacity for mineral uptake compared with plants inoculated with the wild type (Barbieri and Galli, 1993). Emine *et al*. (2006) found that tomato seeds inoculated with *Azospirillum brasilense* FT326 resulted in higher shoot and root fresh weight compared with uninoculated control. The levels of IAA and ethylene, two of the phytohormones related to plant growth, were higher in inoculated plants.

2.4 Leonardite and humic substances

Leonardite is the material formed by the natural decay of a type of lignite coal which contains high humic substances such as humic acid, fulvic acid, and humins as well as other compounds. Leonardite that is very rich in humic acid is applied to plants for use in agriculture. Humic acid is a long chain molecule, which is high in molecular weight, and dark brown in color (Fig. 2.4). It is also soluble in an alkaline solution (Stevenson, 1982).

Figure 2.4 Leonardite powder

The sources of humus, both humic substances and non-humic substances, are various kinds of organic matter such as organic fertilizer and compost including peat and leonardite. Humus can be divided into three types: humic acids, fulvic acids, and humins. Humic substances are composed of the essential elements: carbon, hydrogen, oxygen, nitrogen, and sulfur in complex carbon chains.

Humic acids (Fig. 2.5) are soluble in alkali, can be precipitated by acid, and are insoluble in water. It is a colloid material that has a high molecular weight. The shape is non-amorphus and the particle is 3-10 nm in diameter. The color can vary from yellow to black or brown.

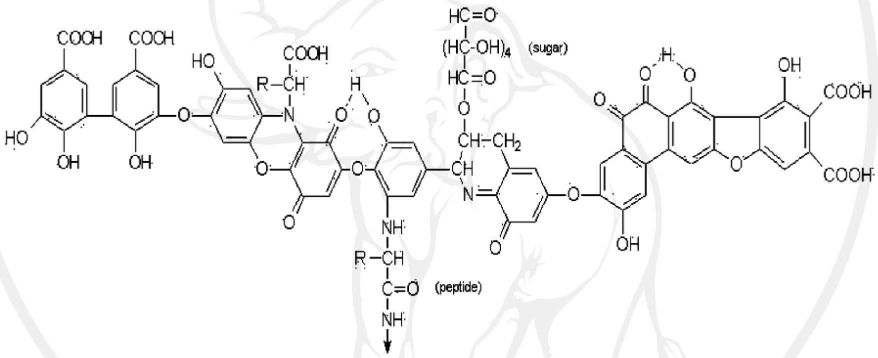


Fig. 2.5 The structural model of humic acid

Within organic matter leonardite contains very high levels of humic and fulvic acids (40-85%) compared to that of peat (10-20%) and compost (2-5%). Humus contained in organic matter is the key factor which promotes soil fertility, crop yield, and microbial activities. Humic substances are an important source of energy for beneficial soil organisms. Humic substances and non-humic (organic) substances provide the energy and minerals required for soil microorganisms. Humic acid can be absorbed into plants via microorganisms. Beneficial soil organisms lack the photosynthetic apparatus to capture energy from the sun and thus must survive on residual carbon containing substances in the soil. Energy stored within the carbon bonds functions to provide energy for the various metabolic reactions within these microorganisms.

Giannouli *et al.* (2009) evaluated the use of Greek peat and coal samples for applications in the agricultural/horticultural sector and assessed the suitability of peat/coal either as soil conditioner or as raw material for manufacturing organic fertilizers. Twenty-six samples of different ranks ranging from peat to subbituminous coal obtained from several Greek peat/coal deposits were studied. The majority of the samples revealed moderate to high ash yields of 16–80 % by weight, a slightly acidic to neutral character, and electrical conductivity ranging from 100–2500 μS/cm. Concerning the environmental impact of the sensitive trace elements, which might be leached, As, Mn, Ni, and Sr showed relatively strong mobilization in some samples, although severe impacts are not expected. The soil's cation exchange capacity was generally improved, although it remained at moderate levels. The most interesting aspect was the humic acid content, which ranged between 9.6 and 52.2% by weight on a dry basis.

Cooper *et al*. (1998) found that humic acid extracted from soil, peat, or leonardite increased the phosphorus content of turf grass by 3-5%. Humic acid from leonardite significantly increased the root weight of turf grass compared to control. Studies have shown that humic substances were able to enhance seed germination of maize, wheat, and barley. Root development of maize was doubled when humic substances were added to the nutrient solution (Kononova and Pankova, 1950; Dixit and Kishore, 1967; Chen and Avid, 1990). Besides the positive effect on plant growth, humic substances also increased nitrogen, phosphorus, and potassium uptake in ryegrass (Dormaar, 1975; Guar, 1964). Humic substances contained plant hormones such as IAA and IAA-like substance and showed phytohormone-like activity (Pizzeghello *et al*., 2001). Levinsky (1997) stated that humates increased the yield and vitamin and sugar content of various crops. Fernandez-escobar *et al.* (1996) reported that foliar application of leonardite extracts to young olive plants stimulated shoot growth when they were growing without the addition of mineral elements to the irrigation water. Under field conditions application of leonardite extracts stimulated shoot growth and promoted the accumulation of K, B, Mg, Ca, and Fe in leaves. However, when leaf N and K values were below the threshold limit for the sufficiency range, foliar application of humic substances was ineffective to promote accumulation of these nutrients in leaves.

2.5 Seedling media

The use of high quality seedling media must be considered as the first priority for increasing vegetable production in the greenhouse. In addition, the development of local high quality seedling media could decrease media imports from abroad, thus promoting the beneficial use of local materials. A report showed that microbial inoculation in vegetable production at the seedling stage increased the vegetable yield, both in shoot and root weight. This also resulted in a higher seedling survival rate after transplanting (Vestberg *et al*., 2004). The use of beneficial microorganisms such as *Azospirillum*, *Beijerinkia*, and actinomycetes is an alternate way to increase the quality and yield of vegetable crops. It also allows the seedlings to have high survival rates after transplanting, which helps promote growth and disease-resistance. This can be extended and applied to improve the soil, productivity, and reduce chemical fertilizer usage as well as the cost of production (Jacoud *et al.*, 1999; Swaminathan and Srinivasan, 2006).

The application of microbial biotechnology together with high quality seedling media can reduce the use of agrochemicals in vegetable production. As a result, this would reduce the toxic contamination of the environment and lead to a better establishment of seedlings after transplanting that would be ensured by a well developed root system (Shutsrirung *et al*., 2009).

Chilembwe *et al.*, (2004) conducted an experiment to evaluate the performance of 5 local substrate composts in raising tobacco seedlings in the nursery. The results showed that pine bark compost and used coal pebbles had the fastest rate of seed germination and total germination percentage followed by coffee husks and ground nut shells. Furthermore, pine bark compost, macadamia husks, and coffee husks had the highest plant growth. Farmers are therefore being encouraged to use the compost of ground nut shells, coffee husks, macadamia husks, and used coal pebbles as growth media when raising tobacco seedlings in the floating tray system. El-Khawas and Adachi (1999) investigated the effect of different concentrations of filter-sterilized culture supernatants of *Azospirillum brasilense* and *Klebsiella pneumoniae* in culture media supplemented with tryptophan on the development of rice roots grown in hydroponic culture medium. The addition of the optimum concentrations (6–8%) of bacterial supernatants to such hydroponic cultures increased root elongation, root surface area, root dry matter, and development of lateral roots and root hairs compared to untreated roots. On the other hand, the addition of high concentrations of the supernatant (more than 10%) strongly inhibited root elongation and lateral root development, and caused root outgrowths, i.e. round nodule-like tumours. Nancy *et al.* (2003) studied the effects of six formulations of plant growth-promoting rhizobacteria (PGPR) added to a soilless, peat-based transplant medium before seeding of muskmelon (*Cucumis melo*) and watermelon (*Citrullus lanatus*). The results showed that PGPR increased shoot weight, shoot length, and stem diameter of both plants. From the above reports, seedling media and beneficial microorganisms are quite important in terms of nutrient uptake and overall growth of the seedlings. The use of seedling media using agricultural wastes that contain high humus materials and the addition of selected efficient microorganisms will not only promote the recycling of agricultural wastes but will also enhance the practical use of biotechnology in sustainable agriculture (Shutsrirung, 2005).

2.6 Chinese kale *(Brassica oleracea* var. alboglabra*)*

Chinese kale (*Brassica oleracea* var. alboglabra), also called Chinese broccoli or Kai-lan, is similar in use and appearance to broccoli. It is a leafy vegetable in the mustard family that has glossy, blue-green leaves with a crisp, thick stem that reaches 7-9 inches tall. Chinese kale is a cool season crop with some frost tolerance but may be grown all year round in temperate and tropical regions. When the plant is still immature Chinese kale is harvested, which is approximately 40 to 50 days after seeding. It is widely eaten in Chinese cuisine, especially Cantonese cuisine. Unlike broccoli where only the flowering parts are eaten, the leaves and also the stem of Chinese kale are edible.

Further enhancements to the nutritional value of Chinese kale by using bio-organic inputs in the production system could result in improved consumer health. High humus materials have the potential to improve not only various soil properties, crop yield, and microbial activities but also the nutritional value of crops. Therefore, in this study, a high humus material (leonardite) was added to organic substrates (coconut husk compost) in various rates with and without beneficial microorganisms to evaluate their effects on nutritional value and yield of Chinese kale.