INTRODUCTION

White horehound (*Marrubium vulgare* L.) is an aromatic medicinal plant belongs to the family *Lamiaceae* and native to Europe, northern Africa and Asia. It is a gray-leaved herbaceous perennial plant, which grows to 25-45 cm height. The leaves are 2-5 cm long with a densely crinkled surface, and are covered in downy hairs. The flowers are white, borne in clusters on the upper part of the main stem (Everist, 1981).

Egyptian Priests called this plant the 'Seed of Horus,' or the 'Bull's Blood,' and the 'Eye of the Star'. It was a principal ingredient in the Caesar's antidote for vegetable poisons. Gerard recommended it, in addition to its uses in cough and cold, to 'those that have drunk poison or have been bitten of serpents,' and it was also administered for mad dogge's biting. It was once regarded as an anti-magical herb. Horehound is a serviceable remedy against Cankerworm in trees, and it is stated that if it is put into fresh milk and set in a place pestered with flies, it will speedily kill them all (Parsons and Cuthbertson, 2001).

Marrubiin is the main active compound isolated from *Marrubium vulgare* L. which used as a medicinal plant in folk medicine to cure several diseases particularly gastroenteric and respiratory **(**Rodrigues *et al.* 1998).

Marrubium valgare L. a medicinal plant employed frequently in folk medicine to treat a variety of ailments, exhibits antispasmodic effects in different experimental models (Jesus *et al*. 2000).

Aqueous extract of *Marrubium vulgare* L. provides a source of natural antioxidants, which inhibit LDL oxidation and enhance reverse cholesterol transport and thus can prevent cardiovascular diseases development. These antioxidant properties increase the anti-atherogenic potential of HDL (Berrougui *et al*. 2006).

Nitrogen is most recognized in plants for its presence in the structure of protein molecule, it plays an important role in synthesis of plant constituents through the action of different enzymes. Potassium is a key essential plant nutrient although it is not a constituent of any plant part and it acts as catalyst for many of the enzymatic processes which are necessary for plant growth. It also regulates the opening and closing of stomata which affect carbon dioxide uptake for photosynthesis.

Biofertilizers are one of the most promising alternatives of supplying nutrients required for the growing plant to substitute chemical fertilizers for safe healthy production for human and the environments, particularly for developing countries. Biofertilizers base generally on altering the rhizospher flora, by seed or soil inoculation, with certain organisms capable of inducing beneficial effect on a compatible host. Biofertilization mainly comprises nitrogen fixers (*i.e Rhizobium, Azotobacter*, *Azospirillum, Azolla* or bule greenalgae), phosphate dissolvers (vesicular – arbuscular – mycorrhizae) and available potassium (*Bacillus* and *Pseudomonas*, which are termed as silicate bacteria). These organisms may affect their host plant by one or more certain microbial processes such as nitrogen fixation, production of growth promoting substances like auxins and gibberellins, organic acids, changing unavailable forms of nutrients into available ones that can be easily assimilated by plant enemies including microbial pathogens, insects and weeds and improving vegetative growth, photosynthetic pigments and chemical composition (Lugenberg *et al*. 1991).

The composition of the essential oil obtained from the dried flowering aerial parts of *Marrubium vulgare* L. (Labiatae) by GC and GC/MS. The major constituents of the essential oil were β-bisabolene (25.4%), β-Caryophyllene (11.6%), Germacrene-D (9.70%) and β-Farnesene (8.9%), (Mahnaz Khanavi *et al*. 2005).

Accumulation of furanic labdane diterpenes marrubiin was investigated in different parts of field grown plants of *M.vulgare*. Furanic labdane diterpenes (marrubiin) were produced and accumulated only in aerial parts. Highest amounts were measured in leaves and flowers (up to 4 mg/gm fresh weight). Accumulation of furanic labdane diterpenes in plantlets seemingly depended on a developments stage. No furanic labdane diterpenes were deleted in plantlets during the first 4 to 5 weeks following germination. After this time the leaves became more differentiated and the number of trichomes on leaves increased. Young leaves and buds contained the most furanic labdane diterpenes, (Knoss and Zapp, 1998).

The objective of this study was to investigate the effect of nitrogen and/or potassium biofertilizers on growth, essential oil yield and constituents and chemical composition of *Marrubium vulgare* L. plants.

REVIEW OF LITERATURE

Organic materials are added to soils to improve their physical and chemical properties. They increase the soil fertility due to their content of macro and micro elements. Organic fertilizers are also considered to be a useful habitat for several beneficial microorganisms. In the presence of organic materials, the number of N-fixing bacteria, phosphorus and potassium solubilizing microorganisms increases in the soil.

Bio-fertilizers consist mainly of beneficial microorganisms that can release nutrient substances from rocks and plants residues in the soil and make them available for economical plants. Therefore, the use of bio- fertilizers is a particular interest to avoid the harmful effect of chemical fertilizers on human health and environment. The beneficial microorganisms were found to have not only the ability to fix nitrogen but also to release certain natural phytohormones of gibberellic and indolic acids, which could stimulate plant absorption of nutrients, photosynthesis processes and plant growth.

Free living N- fixing microorganisms are widely distributed and found in almost every ecological niche in soil associated with plants, in aquatic systems and sediments. Non- symbiotic N- fixed was restricted to certain microorganisms mostly bacteria and blue green algae. N-fixing microorganisms included at least some strains and species of aerobic or microaerophilic *Azotobacter* and *Azospirillum* (Sprent and Sprent, 1990).

Several soil bacteria, particularly those belonging to the genera *Pseudomonas* and *Bacillus* posses the ability to bring insoluble phosphates in soil into soluble forms by secreting organic acids such as formic, acetic, lactic, fumaric and succinic acids. Those acids reduce the pH and bring about the dissolution of bonds forms of phosphate and render them available for growing plants. Effect of free living N-fixing bacteria, phosphate and potassium solubilization bacteria on vegetative growth, yield and chemical composition for some of plants were studied by many investigators. The available literature on this subject will be reviewed as follows:

1. Vegetative growth and yield.

Attia and Saad (2001) on *Catharanthus roseus* plants, fertilized by nitrogen (as ammonium nitrate) at 0, 28, 56 and 84 kg N/fed., and bioferilizer (nitrobein) at 250 g/fed., found that the low rate of nitrogen (28kg/fed) with nitrobein gave the best results of plant height, number of branches and herb fresh and dry weights per plant.

El-Kashlan (2001) on roselle plants recorded significant increases in plant height and number of branches/plant. There is an increase in the number of fruits and plant height and fresh and dry weights of sepals as a result of using three commercial bio- fertilizers (Biogene, Nitrobien and Phosphorene).

Gad (2001) found a significant increase in plant height, number of leaves, fresh and dry weights of vegetative growth, number of umbels and fruit weight/plant as a result of using biofertilizers on *Foeniculum vulgare* and *Anethum graveolens*.

Gomaa and Abou-Aly (2001) studied the effect of inoculation with non- symbiotic N_2-fixers (*Azotobacter chroococcum* and *Azospirillum brasilense*) in the presence of biogas manure or inorganic nitrogen fertilizer on growth of anise plants. The results showed that Cl_2 evolution and nitrogenase activity of anise plant rhizosphere were the highest with the treatment of *Azotobacter chroococcum* with the addition of either biogas manure or inorganic nitrogen fertilizer. Also, inoculation of anise seeds with *Azotobacter* or *Azospirillum* in the presence of biogas manure recorded the highest values of *Azotobacter* and *Azospirillum* densities. They also revealed that there was a significant increase in vegetative growth of anise plants due to inoculation with non- symbiotic N_2- fixers and biogas manure.

Abd El- Latif *et al.* (2002) studied the effect of different levels of chemical fertilizer (0,200 and 400 Kg/fed. ammonium sulphate) and bio - nitrogen fertilizer (1, 2 and 4 Kg/fed. biogen) on growth and yield of *Matricaria chamomilla*. They found that bio- nitrogen fertilizer produced enhancement in growth characteristics and yield particularly at the highest rate (4 Kg/fed. biogen). The best results were recorded with the combination between ammonium sulphate at 200 kg/fed., and biogen at 2 and 4 kg/fed.

Abd El Latif (2002) on *Carum carvi*, noticed that nitrobein at the rate of one kg/fed., produced increase in plant height, number of branches and fresh and dry weights per plant.

Abdou and El- Sayed (2002) on *Carum carvi*, found that the biofertilization treatment (*Azotobacter chroococcum* and *Azospirillum* *lipoferum*) at 600 g/fed., was effective on stimulating growth traits (plant height, number of branches and stem diameter).

Abo El- Ala (2002) studied the effect of inoculation with the mixed three most efficient strains of *Azotobacter chroococcum, Azospirillum lipoferum* and *Bacillus megatherium* on the growth and yield of *Ocimum basilicum* and *Majorana hortensis* in the presence of different carriers (sodium alginate and peat) in the presence of 2 doses of N and P fertilization: normal field dose (150 kg calcium ammonium nitrate and 300 kg calcium superphosphate/fed) and half of these amounts. He found that uninoculated plants provided with the full dose of inorganic N and P fertilizers gave plants of less height, fresh and dry weights than those inoculated with the multi- biofertilizer inoculants based on alginate in the presence of half normal field dose of inorganic N and P fertilizers. Amended plants with the half dose of N and P fertilizers and inoculated with the multi- biofertilizer inoculant based on alginate gave the highest number of branches after 140 days from cultivation.

Abou- Aly and Gomaa (2002) stated that inoculating coriander seeds with *Azotobacter chroococcum* and *Bacillus megatherium* var. phosphaticum significantly increased plant height, plant fresh and dry weights, number of leaves and branches per plant, root length and fresh and dry weights of root per plant.

Dessouky (2002) studied the effect of nitrobein at rate of 0 and 5kg/fed., on *Borago officinalis*. He found that the rate of nitrobein (5 kg/fed) was the most effective treatment on seed yield compared with control, as well as the plant height, number of branches and fresh and dry weights of the herb.

Gomma (2002) on *Coriandrum sativum* and *Majorana hortensis*, observed that applying active dry yeast at 10 g/L as a soil drench and full dose of NPK plus biofertilizer (a mixture of *Bacillus megatherium, Azospirillum lipoferum* and *Azotobacter chroococcum*) gave the best vegetative growth parameters as plant height, number of branches/ plant, fresh and dry weights of vegetative growth/ plant, number of total umbels/ plant, weight of 100 seeds and seeds weight/plant.

EL-Ghadban *et al*. (2003) studied the effect of organic fertilizer and biofertilization on marjoram plants. They found that using compost at 5 or 7.5 ton/fed., in combination with the mixture of *Azotobacter chroococcum, Azospirillum brasilense* and *Bacillus polymyxa* increased plant height, number of branches and herb fresh and dry weights.

Kandeel and Sharaf (2003) showed that *Azotobacter chroococcum* produced an increase in plant height, number of branches, and fresh and dry weights of *Majorana hortensis* L. plants.

Hafez (2003) on borage, stated that nitrobein (55% *Azotobacter chroococcum* and 45% *Azospirillum lipoferum* containing one gram on 10^7 cell) at 600 g/kg seeds enhanced greatly plant height, number of branches, leaves fresh and dry weights and branches fresh and dry weights, compared with the control.

Mahfouz (2003) studied the effect of biofertilization on *Majorana hortensis* using a mixture of 5 strains namely: *Azotobacter* *chroococcum, Azospirillum lipoferum, Bacillus polymixa, Bacillus megatherium* and *Pseudomonas fluorescence* in the presence of full dose of NP fertilization (300 kg ammonium sulphate and 200kg calcium superphosphate/fed) and half dose of NP fertilization. He found that the highest values of vegetative growth were recorded at the treatment of biofrtilizer plus full dose of N and P than the other treatments and control plants. Inoculating the plants in the absence of N and P, half dose of N with inoculation, half dose of N and full dose of P with inoculation increased growth parameters and yield compared to control plants.

Annamalai *et al*. (2004) on *Phyllanthus amarus* plant, found that the maximum shoot length, root length, branches per plant, leaves/ branch, fruits/ branch and fresh and dry weights obtained with 15 t farm yard manure/ ha treatment. The highest numbers of compound branches/ plant and seed yield / plant obtained with 12 t farm yard manure + 2.5 kg *Azospirillum* + 2.5 kg phosphobacteria / ha treatment.

Al- Humaid (2004) on fennel, reported that inoculation with *Rhizobacteria* significantly increased plant height, number of umbels, fresh and dry weights of whole plant and fruits yield per plant and per fed.

Kandeel *et al*. (2004) recorded a significant increase in plant height, number of leaves, fresh and dry weights of vegetative growth, number of branches, number of umbels and fruits weight/ plant as a result of using bio- fertilizers (Biogene, Netrobene and Serialene) on *Anethum graveolens* and *Foeniculum vulgare*.

Migahed *et al*. (2004) inoculated *Apium graveolens* plants with nitrogen fixing bacteria (*Azotobacter chroococcum* and *Azospirillum lipoferum*) and phosphate- solubilizing bacteria, (*Bacillus megatherium*) as bioferilizer applied singly or in combination and with or without gibberellin at 200 and 400 ppm. The highest plant growth such as plant height, branches, umbels, fresh and dry weights / plant of celery was obtained in *Azotobacter* followed by *Azospirillum* and then *Bacillus megatheriem*.

Hamed (2004) on *Salvia officinalis* and *Origanum syriacum*, stated that plants fertilized with ammonium sulphate (600 and 400 kg, respectively) + calcium superphosphate (200 and 133 kg, respectively)+ potassium sulphate (100 and 66 kg, respectively)/ fed./year and inoculated with a biofertilizer (1 L/fed. mixture of 5 strains of bacteria namely: *Azotobacter chroococcum*; *Azospirillum lipoferum*; *Bacillus polymixa*; *Bacillus megatherium* and *Pseudomonas fluorescence* + 20 L of water/ fed) increased plant height, number of branches/ plant and fresh and dry weights of herb/plant.

Zayed *et al.* (2004) investigated the effect of ammonium sulphate applied at 0, 100, 200 and 300 kg/fed., and nitrobein at 0, 200, 400 and 600 g/kg seeds on the growth of *Borago officinalis*, L. They found that N fertilizer application increased the seed yield by increasing the rate to 200 kg ammonium suphate/fed. Moreover, treating borage plants with nitrobein increased the seed yield especially at the rate of 600 g/kg seeds compared with the other treatments.

Sakr (2005) treated *Cassia acutifolia* Delile, plants growing in sandy soil with different treatments of organic manure (poultry10m^3

and cattle 20m^3/fed) and biofertilizer (a liquid culture of compost inocula of *Azospirillum brasilense Baicllus polymyxa*, *Azotobacter chroococcum*, *Klebsiella pneumonia* and *Pseudomonas putida* a liquid inoculum = 16^8 cell/ml). Plants were harvested twice/ season all treatments increased plant height, number of leaves, herb fresh and dry weights / plant (leaves and pods).

Shaalan (2005-a) on *Borago officinalis* L plant, showed that biofertilizer application plus organic manure improved plant growth characteristics expressed as plant height, number of branches, fresh and dry weights/ plant and per plot of both leaves and flowers, seed yield, fixed oil percentage and delta- linolenic acid of different treatments. The best results were obtained by inoculation of plants with nitrobein plus phosphorein and organic manure at rate of 24 m^3/ feddan.

Shaalan (2005-b) inoculated the seeds of *Nigella sativa* L. with *Azotobacter sp.* (Biogein), *Azospirillum sp.* (Nitrobein) or *Bacillus megatherium* (Phosphorein) each of them individually or in combination at a rate of 7 kg/fed., for each bio-fertilizer. He postulated that all bio-fertilizer treatments increased plant height and number of branches per plant, but the most effective treatment was the mixture among all biofertilizers.

Meenakshi Banerjee *et al*. (2006) on *Catharanthus roseus* and *Adhatoda vasica* plants reported that, inoculated plants with nitrogen fixing cyanobacteria from rice fields as biofertilizers resulted the great enhancement in all parameters of herbal drug like leaves, stems and roots were observed for both plants.

Mostafa (2006) studied the effect of three chemical fertilizers treatments: ammonium sulphate (300kg), calcium superphosphate (150kg) or ammonium sulphate + calcium superphosphate (150+75kg/fed) and three biofertilizers treatments; *Bacillus*, *Azotobacter* +*Azospirillum* or *Bacillus*+ *Azotobacter*+ *Azospirillum* on growth and yield of chamomile plant (*Matricaria chamomilla* L), he found that the treatments of chemical and biofertilizers increased the vegetative growth compared to control treatment. The best results were obtained with combined mineral fertilizers and the three strains of bacteria.

Aly *et al*. (2007) studied the effect of ammonium sulphate at 0, 150 and 300 kg/fed., calcium superphosphate at 0, 100 and 200 kg/fed., and potassium sulphate at 0,50 and 100 kg/fed., biofertilization; *Azospirillum lipoferum*, *Azotobacter chroococcum* or *Bacillus polymyxa* (each separately or in combination) on *Hibiscus sabdariffa*. L. They pointed out that the medium rate of different mineral fertilizers with mixture of *Azospirillum lipoferium*+ *Azotobacter chroococcum*+ *Bacillus polymyxa* increased number and fresh weight of fruits/plant, sepals fresh and dry weights per/plant and seed yield per plant and per feddan.

Khalid *et al*. (2007) on *Ruta graveolens* L plants, found that fertilized the plant by natural products as a source of important elements such as rock phosphate and feldspar mica as source of potassium or biological potassium fertilizer (silicate bacterium) at different levels (0.0, 25, 50 and 100 g/L. Adding biological fertilizer with feldspar or rock phosphate improved vegetative growth characteristics such as plant height branches number/plant, fresh and dry weights of different plant parts, i.e. leaves, stems and roots (g/ plant).

Kuntal Das *et al*. (2007) treated *Stevia rebaudiana* Bert, with N, P and K as sole and in combinations and biofertilizers (Vesicular Arbuscular Mycorrhiza, phosphorus solubilizing bacteria and *Azospirillum* as sole and in combinations. The results showed that the biomass yield as well as nutrient content in stevia have been recorded an increase significantly due to inorganic chemical and biofertilizer applications. The magnitude of such increase in biomass yield and nutrient content significantly higher with the application of biofertilizer, being further greater increase with combined applications of VAM, PSB and *Azospirillum*.

Mahfouz and Shams-Eldin (2007) on *Foeniculum vulgare* Mill, found that application of biofertilizer, which was a mixture of *Azotobacter chroococcum*, *Azospirillum liboferum*, and *Bacillus megatherium* applied with 50% recommended dose of NPK increased vegetative growth (plant height and herb fresh and dry weights / plant) compared to chemical fertilizer treatments only.

Ismail (2007) on dragonhead *Dracocephalum moldavica* plant, found that the highest weight of the aerial part was obtained from 1/4 Min + 1/4 Org+ Bio treatment, while the highest leaves and flowers production were obtained from 1/2 Min+ Bio treatment.

Abdel- Ghani (2008) on *Rosmarinus officinalis* L, found that nitrobien as N-bioferlilzer (N fixing bacteria) at 3 and 6 g/plant significantly increased plant height and fresh and dry weights/ plant compared to control, whereas nitrobien at 6 g/plant treatment gave the highest values of No. flowers/ plant and fresh weight of herb of *Matericaria chamomilla* L compared to control.

Gharib, *et al.* (2008) on *Majorana hortensis*, found that the use of combined treatment of biofertilizers [compost+ mixture of N fixers (*Azospirillum brasiliense*, *Azotobactor chroococcum* and *Bacillus polymyxa* and *Bacillus circlans*)] gave the best results for all growth parameters than those obtained from N fixers or *Bacillus circulans* alone.

Hassan (2009) on *Hibiscus sabdariffa* L plant, found that using Easterna biofert or bacteria inoculation separately or combined with chemical fertilizers significant improved growth characteristics and increased sepal yield of roselle plant compared to the control.

On the other side, Matter (2009) showed that, inoculation of roselle seeds with *Rhizobium* and *Azotobacter* alone significantly decreased plant height, number of branches, number of leaves, leaf area, dry weight of leaves, number of flowers per plant and fresh and dry weights of sepals per plant and per fed., compared to control plants during the two successive seasons.

Ezz El-Din and Hendawy (2010) reported that fertilization of *Borago officinalis* with compost tea at 20 L/fed. significantly increased plant height, number of branches, fresh and dry weights of aerial parts and flowers.

Hendawy *et al.* (2010) found that fertilization of *Thymus vulgaris* L. with 20 m^3 compost/fed combined with 10 L/ fed of compost tea and /or feldspar, rock phosphate at the level of 150 kg/fed gave superior growth and yield characteristics.

Badi *et al.* (2011) on saffron (*Crocus sativus* L.) plants, found that 100g/ha biofertilizer as biophosphore was the best treatment in respect of fresh stigma and style length, number and leaf length, saffron yield (dry weight of stigma and style) and content of safranal, crocine and picrocrocine.

Darzi *et al.* (2011) on *Pimpinella anisum* L plant, reported that the highest plant height, umbel No/ plant, biological yield and seed yield were obtained from consumption of 10 ton/ha vermicompost but 1000 fruit- weight was not significantly affected by vermicompost, Phosphate bifertlilzer showed significant effects on umbel No./plant, biological yield and seed yield of anise plant.

Rajamanickam *et al*. (2011) treated *Mentha arvensis* plants with organic manures consortium of biofertilizers and inorganic fertilizers, the results revealed that the application of 75% NPK+ vermicompost+ consortium of biofertilizers significantly increased the growth and yield of mint herb.

Hellal *et al*. (2011) investigated the effect of partial substituation of mineral nitrogen fertilizer by biofertilizer on the growth and yield of dill plant during the two successive seasons. Five strains of bacteria *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus polymyxa*, *Bacillus megatherium* and *Pseudomonas fluorescens* were mixed in equal parts and used as biofertilizer. The biofertilizer treatment was applied alone or in combination with 1/3, 2/3 or full recommended dose of mineral nitrogen fertilizer. The results