We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Prospects of N Fertilization in Medicinal Plants Cultivation

Felix Nchu, Yonela Matanzima and
Charles P. Laubscher

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.68165>

Abstract

High global demand for plant-derived medicines is threatening the existence of many wild indigenous plant species. However, the high demand of medicinal plants has also created huge business opportunities in commercial farming of medicinal plants. Large-scale production of secondary metabolites by plants and medicinal materials will be crucial in the medicinal plant industry. As commercial cultivation of medicinal plants gains traction among farmers, N fertilizers will be increasingly used to enhance plant growth and yield. Therefore, the implementation of better nitrogen use efficiency is critically important. Excessive use of N can lead to many problems; it is costly, it can cause environmental pollution and its high levels in plant tissues can be toxic to plants, herbivores and humans. This chapter discusses the potential risks, opportunities and setbacks associated with the use of N in cultivation of medicinal plants.

Keywords: nitrogen fertilizer, medicinal plants, toxicity, yield, secondary metabolite

---

1. Introduction

Exploitation of plant resources for the treatment of human and animal diseases has placed significant pressure on plant biodiversity. It has been reported that more than 3.5 billion people in the developing world rely on plants as components of their primary health care [1]. However, the use of medicinal plants is not only limited to the developing world, in fact demand for herbal medicine is also rising in many developed countries, for example, in Germany, it is estimated that 600–700 plant-based medicines are available and prescribed by 70% of physicians [2]. The demand for plant-derived medicines has created a large business in indigenous plants in South Africa, which is estimated to be worth R270 million annually [3, 4].

In South Africa alone, there are some 27 million indigenous medicinal consumers [5]. The well-known examples of plant species that are currently traded in South Africa include *Artemisia afra* (Asteraceae), *Melianthus comosus* (Melianthaceae), *Aloe ferox* (Asphodelaceae), *Aloe arborescens* (Asphodelaceae), *Salvia Africana-caerulea* (Lamiaceae) and *Helichrysum cymosum* (Asteraceae) [6].

Plant parts obtained from single or varied species are used to prepare medicinal products. Medicinal plant parts contain bioactive principles that are often referred to as secondary metabolites. Primary metabolites such as enzymes and proteins, lipids, chlorophyll and carbohydrates are fundamental to the life of the plant, while secondary metabolites (terpenoids, the alkaloids, the phenylpropanoid and some phenolic compounds) do not appear to be necessary to sustain life at a fundamental biochemical level. However, secondary metabolites play important defence and chemical ecological roles [7]. Medicinal properties can be obtained from the following plant parts: leaves, bulbs, essential oil, fatty acid, flowers, fruit, gum, stem, roots, rhizome, seed, tuber and wood. Plant secondary metabolites are thought to be responsible for the antimicrobial, antioxidant, anti-inflammatory and insecticidal activities of plant extracts [8]. These plant-derived extracts and compounds are exploited for the treatment of human and animal diseases. Large-scale production of secondary metabolites by plants is crucial in the medicinal plant industry. However, the production of secondary metabolites by plant depends on endogenous and exogenous factors [9]. Nitrogen is one of the most important nutrients needed by plants for growth. Information on the role of nitrogen in plant physiology is plentiful in literature. Nitrogen is involved in many physiological processes in plants including growth and photosynthesis. Consequently, nitrogenous fertilizers are among the most used fertilizers in the world. Nevertheless, excessive use of N can have negative economic and the environmental implications. Intensive N fertilization can lead to toxic N levels in plant tissues and herbivores. Thus, there are calls for implementation of better nitrogen use efficiency (NUE) [10].

Researchers have recognized the potential benefits of manipulating nutrient nitrogen supply for optimal plant growth and the need to minimize some of the setbacks associated with nitrogen fertilization. This has incentivized the quest for the development of precision fertilization and innovative plant cultivations methods. For examples, the use of sustainable, innovative and precision agronomic technologies such as hydroponics, aeroponics, aquaponics and organic farming can optimize the manufacturing of natural molecules of pharmaceutical and cosmetic significance. According to Masclaux-Daubresse et al. [10], increasing nitrogen use efficiency in the contexts of plant nutrition and limiting nitrogen fertilizer use is important. It is essential to preserve the environment, while promoting sustainable and productive agriculture. Therefore, knowledge on nitrogen availability and conservation in growth media, and nitrogen uptake, assimilation and translocation by plant are critically important to the development of efficient nitrogen fertilization strategies. This chapter discusses the potential risks, opportunities and setbacks associated with the use of N in cultivation of medicinal plants.

2. Demand for medicinal plants and rationale for commercial cultivation

Until recently, the most commercial farmers have been focused on improving quality and quantity of agricultural and horticultural crops over medicinal plants. Medicinal plants are

used in traditional practices worldwide and their use has been increasing steadily. Medicinal plants constitute an important component of health care systems, globally. The trade of medicinal plants is estimated to be worth R270 million annually [3]. According to Sobiecke [11], globally, products that are derived from traditional medicine are estimated to be worth R2.9 billion per year. On the demand for medicinal plants is increasing worldwide and it is estimated up to 700,000 tonnes of plant material are consumed annually to the value of about 150 million US dollars [4]. The World Health Organization estimates that 21,000 species are used for medicinal purposes around the world and in India 150 species are used commercially [12]. In Zimbabwe, herbal medicine is the most affordable and easily accessible form of treatment in primary health care and up to 93 medicinal plant species are used in the south-central region of Zimbabwe [13]. In Pakistan, more than 500 species of plants are used in herbal medicine [14]. Street and Prinsloo [15] presented 10 highly used South African medicinal plants, such as *Agathosma betulina* (Rutaceae), *A. ferox* (Asphodelaceae), *Aspalathus linearis* (Fabaceae), *Harpagophytum procumbens* (Pedaliaceae), *Hypoxis hemerocallidea* (Hypoxidaceae), *Merwillia natalensis* (Hyacinthaceae), *Pelargonium sidoides* (Geraniaceae), *Siphonochilus aethiopicus* (Zingiberaceae) and *Sutherlandia frutescens* (Fabaceae) in a review paper. Although some critics have argued that traditionally it is not acceptable to use cultivated medicinal plants, a recent report on the perception of cultivation of medicinal plant species indicated that very high proportions (over 69%) of respondents are willing to buy and make use of cultivated medicinal plants [16]. This trend suggests that developing efficient and sustainable agro-technology should be one of the focal areas for research.

Cultivation of medicinal plants is gaining momentum among subsistence and commercial farmers [17]. Farming of medicinal plants has many advantages, for examples it can contribute to job creation and improvement of household earnings, and it can reduce over-exploitation and harvesting of some wild and endangered species. Similar to the cultivation of food crops, medicinal plant cultivation programmes should have specific goals, which include to increase medicinal plant yield and plant growth rate, increase and standardized quality and quality of secondary metabolites produced and reduce toxicity to humans. It is worth noting that commercial cultivation may inadvertently lead to environmental degradation and loss of genetic diversity as well as loss of incentives to conserve wild populations [18]. However, Wiersum et al. [4] argued that the impact of the cultivation of medicinal plant can be beneficial if it is done within the context of protecting and strengthening the cultural values of biodiversity and creating a positive attitude towards biodiversity conservation in general.

3. Nutrient nitrogen

Nitrogen is one of the most important nutrients needed by plants; it is an important element for the formation amino acids, it is essential for plant cell division, it is directly involved in photosynthesis, it is an important component of vitamins and it aids in the production of carbohydrates. Physiologically, N is mostly available to plants in the forms of ammonium and nitrate and preference for one of the two forms to be taken up by plants tend to be influenced by the plant species and soil conditions, including pH and soil temperatures [10, 19].

Nitrate uptake is followed by reduction to nitrite, which is then transported to the chloroplast wherein it is reduced to ammonium and is mostly assimilated in the plastid/chloroplast and finally undergoes nitrogen remobilization, whereby leaf proteins and especially photosynthetic proteins of plastids are extensively degraded during senescence, providing an enormous source of nitrogen that plants can tap to supplement the nutrition of growing organs such as new leaves and seeds [10]. Nitrogen is available to plants from varied sources and includes inorganic fertilizers (ammonium nitrate, ammonium sulphate, urea, calcium ammonium nitrate and diammonium phosphate and sodium nitrate), organic (compost, manure, seaweed, fish meal and fish emulsion and guano) sources. Although nitrogen occurs naturally in soils, generally, the quantity is quite low and varies geographically warranting external N inputs in the form of fertilizers.

Both organic and inorganic N fertilizers have advantages and disadvantages. Inorganic fertilizers provide readily available nitrogen; however, they are easily lost by leaching, denitrification, volatilization and run-off. Furthermore, inorganic fertilizers have been frequently linked to cases of environmental contamination, soil acidification and salinity. On the other hand, organic fertilizers release of N to plant tends to be slower and depends on the mineralization rates. Nevertheless, organic fertilizers improve the soil physical and chemical properties. Some of the setbacks associated with the use of organic or inorganic fertilizers are predominant in plant cultivation whereby the growth medium is soil. Inherent variations in biophysicochemical properties of soils make it difficult to accurately determine the effects of fertilization on plant growth, yield and quality of produce. Factors such as seasonal changes, development stages, levels of pathogens, geographical differences and nutrient status of the soil affect the amount of secondary metabolites plants produce [20, 21]. These factors can potentially influence the standardization of the quality of medicinal materials. Consequently, more precise plant cultivation techniques are increasingly being used in crop cultivation.

According to Jehnson [22] and Hayden [23], hydroponics technology is a technique of growing plants in a nutrient solution (water and fertilizers) with or without the use of artificial medium (e.g. sand, rockwool, vermiculite, gravel, peat moss, coir and sawdust) to provide a mechanism of support. The advantages of using hydroponics include high-density maximum crop yield, crop production can be achieved in areas where good soil for production is not available, plants can be grown during off-season and temperature can be manipulated [22, 24]. In hydroponics, N is supplied to plants in the form of dissolved salts, which is usually prepared in small and precise quantities, and different nutrient recipes and combinations can be used. Hydroponic technology can be used to manipulate production of plant secondary metabolites [25]. It can favour plant vigour, decrease poisonous levels of plant toxins, increase uniformity and probability of obtaining bioactive extracts [26]. Other related technologies such as aquaponics and aeroponics can also be used to cultivate some medicinal plant species; however, they are still to be fully explored. Aquaponics is the combination of hydroponics and aquaculture in an integrated system to raise fish and grow plants, simultaneously, while aeroponics is a liquid hydroponics system with no other supporting medium for the roots of the plants [22]. In aeroponics plants are grown in misty environment.

4. Physiological effect of nitrogen on medicinal plants

Fertilization programme in medicinal plants has two important objectives: high vegetative growth and high quantity and quality of secondary metabolites produced. Meeting these objectives could lead to high medicinal materials and increased medicinal value of a plant. Generally, N supply favour increased vegetative growth. Argyropoulou et al. [27] investigated the effect of nitrogen starvation on morphological, physiological and biochemical parameters of basil plants cultivated aeroponically. They observed that net photosynthesis rate, transpiration rate, the stomatal conductance and the concentration of total chlorophylls were strongly restricted by N deprivation rate and that total phenolic concentration significantly increased in N-starved plants indicating that biosynthesis of secondary plant metabolites is favoured in nitrogen-deficient plants. Periwinkle, a medicinal plant that is rich in terpenoid alkaloids, when exposed to mixture of nitrate and ammonium, produced the highest content of amino acids, proteins, total alkaloids, vincristine and vinblastine compared to each of the different N forms. It was also observed in the same study that increase in N level beyond 11 mM had an antagonistic effect on alkaloid content [28]. Previous studies have indicated that when plants have N deficiency they tend to have increased concentration of C-based secondary metabolites [29, 30]. Future studies that identify critical N levels for important medicinal plant species will guaranty both high production of medicinal material and quantity and quality of bioactive medicinal principles.

5. Nutrient nitrogen threshold

Nitrogen is a major constituent of enzymes, proteins, chlorophyll and is involved in many important biochemical processes in plants including photosynthesis. However, it has been shown in many studies that N effects on plant physiological processes like syntheses of amino acids and phenolics are dependent on tissue N concentration, plant species and other exogenous factors like water availability, temperature and light. Yañez-Mansilla et al. [31] hypothesized that there is an optimum N concentration threshold that ensure a high phenolic concentration and antioxidant capacity without detrimental effects on plant performance and proposed a threshold of 15 g N/kg DW as an optimum concentration for ensuring high antioxidant activity and quality in blueberry leaves, based on results obtained in their study. In order to meet requirements of new regulations in the coastal valleys of central California, USA, field trials were carried out by Bottoms [32] to identify commercial fields in which N application could be reduced or eliminated in order to improve nitrogen (N) fertilizer efficiency. Crop growth, N uptake and the value of soil and plant N diagnostic measures were evaluated in 24 iceberg and romaine lettuce plants and it was concluded that soil $\text{NO}_3\text{-N}$ greater than 20 mg/kg was a reliable indicator that N application could be reduced or delayed. Many farmers, scientists, consumers and governments are becoming aware of the risks associated with excessive nitrogen fertilization and are seeking environmentally friendly and sustainable approaches of N fertilization. Medicinal crops farmers would have to take cognizance of the need to balance high yield, quality medicinal materials and minimum environmental toxicity. It is expected

that indigenous plant species, especially those occurring in their natural habitats are adapted to their local conditions and may tend to have low critical levels for most of the nutrients. For example, medicinal plants occurring in the fynbos biome of South Africa are adapted to nutrient-poor and low pH soils. Therefore, exposing these species to high N concentration may have minimal effect on plant physiology and can even have detrimental effects on plant growth.

6. Economics of nitrogen fertilization

Many studies have demonstrated that plant yield increases with N fertilization. The quest by farmers for high yield and high profit margins has encouraged the implementations of inappropriate N fertilization programmes. Excessive and inadequate N supply to plants could induce deleterious effects in plants and the environment. With increasing N fertilization costs, it is important to determine optimum N fertilization rates in order to achieve economically viable N fertilization in crop production. In a study carried out in Viçosa, Minas Gerais State, Brazil that aimed at determining the economic optimum N fertilization rates under cold and ambient conditions of four potato cultivars, it was found that economic optimum N fertilization rates ranged from 147 to 201 kg/ha depending upon cultivar and relative prices of N and potato tubers [33]. Farquharson et al. [34] recognized the importance of environmental effects such as N_2O emission of N fertilization in Australian wheat production and using an economic framework model, they predicted that the best fertilizer decision is reduced by about 4 kg N/ha (5%) when the Intergovernmental Panel on Climate Change (IPCC)-based environmental cost of N fertilizer is considered. Nyborg et al. [35] reported that economics of nitrogen fertilization of barley and rapeseed is influenced by nitrate-nitrogen level in the soil and suggested that soil testing to determine N_2O -N levels is essential for maximum economic returns from N fertilization. Based on the above-mentioned arguments, the use of precision N fertilization approach is encouraged, for example, in hydroponics it is possible to manipulate plants to produce higher yields of bioactive fractions [36].

7. Case study

Preliminary assessment of the effects of nutrient nitrogen on growth and antimicrobial activities of *H. cymosum* grown under greenhouse conditions.

7.1. Introduction

H. cymosum subsp. *cymosum* (Asteraceae) is an indigenous South African medicinal plant (Figure 1). It has high medicinal value and is heavily harvested from the wild. This species is distributed along the coastal areas of the Eastern and Western Cape Provinces. The soil of the coastal region of the Western Cape region is typically acidic and nutrient-poor and is derived from the weathering of granite [37]. The objective of this study was to assess the effect of N fertilization on growth, tissue nutrient content and antimicrobial activities of acetone leaf extracts of *H. cymosum* cultivated on field collected soil samples under greenhouse conditions.

Figure 1. Hydroponics cultivation of the medicinal plant species *H. cymosum* in a greenhouse.

7.2. Materials and methods

Soil was collected from a commercial vegetable farm located in Kuilsriver, Western Cape, South Africa and the soil subsamples analysed (physico-chemical analysis) [38]. The field collected soil was used to prepare 3 kg potted soil samples. Ammonium nitrate salt was dissolved in 500 ml of sterile distilled water and the solution was poured into the potted soils to obtain a final soil concentration of nitrogen that was 136 ppm. Potted soils were placed in rows on a steel table. In the control treatment, only 500 ml of sterile distilled was added and the baseline N concentration was 32 ppm. Six weeks old rooted cuttings of *H. cymosum* were transplanted individually into each pot. A total of 16 pots, grouped into two treatments with eight replicates per treatment were used. Parameters such as plant height, nutrient concentration of leaves and leaf numbers were assessed in order to determine the effects of nitrogen and potassium on growth of *H. cymosum* at the end of the experiment, 13 weeks post-treatment. Leaf tissue analysis was carried out [39, 40]. Fresh foliage harvested at 13 weeks post-treatment was air dried at room temperature for 4 weeks. Dried plant materials were cut into smaller pieces and ground using a Jankel and Kunkel Model A 10 mill into fine powder. Powdered leaf material (5 g) was extracted with 100 ml of acetone in a glass beaker with the aid of a vortex mixer for 15 min and the supernatant filtered using Whatman No.1 filter paper. The extracted material was left to dry overnight. The micro-dilution method previously described by Eloff [41] was employed with slight modifications to determine the minimum inhibitory concentration (MIC) for the extracts. *Fusarium oxysporum* fungal culture was introduced to all microplates (10^5 spores/ml). Mancozeb (60 mg/10 ml) was prepared using sterile distilled water as a positive control and a mixture of sterile distilled water and acetone was used as a negative control. Data were analysed using a one-way analysis of variance (ANOVA).

7.3. Results

There was no significant difference ($P > 0.05$) in plant height exposed to higher level of N (51.4 ± 4.9 cm) compared to those exposed to low level N (Control) (55.1 ± 5.1 cm) at 13 weeks post-treatment. Similarly, no significant difference ($P < 0.05$) was observed in the number of branches in plants exposed to the different N treatments. Comparatively, N-treated (1.9 ± 0.2 ppm) plants had a significantly high levels of tissue content N in the leaves (df 1,6; $F = 7.8$; $P = 0.03$) than those exposed to low nutrient N treatment (1.4 ± 0.1 ppm) at 13 weeks post-treatment (**Table 1**). MIC bioassay did not show a significant effect ($P > 0.05$) on antifungal activity following N treatment compared to control (0.187 mg/ml) (**Table 2**).

7.4. Discussion

Nitrogen-treated plants had higher N content in the leaves compared to low N-exposed plants suggesting that the treatment with an increased level of N could have induced high uptake of nitrogen. The plant growth was not significantly different in plants treated with 136 ppm of nitrogen compared to control plants (32 ppm). This result suggests that higher nitrogen supply may not always result in high vegetative growth. A plausible explanation could be that plants occurring naturally in nutrient-poor area may have low optimum nutrient requirement and may not warrant excessive N treatment. Also, high N fertilization of medicinal plants may not necessarily reduce bioactivity of their extracts.

Treatment	N content ppm
N	1.4 ± 0.1
Control	1.9 ± 0.2

Table 1. Tissue nutrient content (ppm) in aerial parts of *H. cymosum* following exposure to control and N treated field collected soil samples after 13 weeks post-treatment.

Acetone extracts	Minimum inhibitory concentration (MIC mg/ml) of acetone extract of <i>Helichrysum cymosum</i> against <i>Fusarium oxysporum</i>	
	24 h	48 h
N	0.82 ± 0.01	0.187 ± 0
Control	0.93 ± 0	0.187 ± 0

Table 2. MIC antifungal activity of the acetone extract of *H. cymosum*.

8. Nitrogen toxicity

Excessive nitrate fertilization can induce high accumulation of nitrates in plant tissues to levels that are potentially toxic to humans and livestock. However, Qiu et al. [42] showed that

that genotypic variation in nitrate accumulation is associated with differences in water content for rape, Chinese cabbage and spinach. Vegetables account for over 70% of the total nitrogen intake of humans [43]. Increased concentration of nitrite and nitrates in diet are risk factors for many diseases in mammals [44]. Although nitrate intake from vegetables is receiving substantial attention, it is important that cultivated medicinal plants receive similar attention as the industry develops. Commercial cultivation of medicinal plants could lead to excessive N fertilization and high concentration of nitrites and nitrates in medicinal plant parts and subsequently in herbal decoctions and infusions. This can negate the beneficial effects of medicinal plants. Also, accumulation of unused nitrates in soils could have unfavourable effect on soil biological, physical and chemical properties. Furthermore, leached nitrates in water runoffs could lead to eutrophication of freshwater resources. Since plants have different N needs/requirements, research on the N requirement of each plant in different growing conditions is important in order to achieve high yield, safe and good quality medicinal materials from plants.

9. Regulation of N fertilization

The development of fertilization policies in many countries is an indication of recognition of the risk that is associated with the use of fertilizers including nitrogenous fertilizers. One of the main challenges facing regulation of the use of fertilizer inputs include high variations of rate of N fertilization across regions and crops and the stage of economic development [45]. The increasing demand for efficient fertilizer use has led the United Nations Economic Commission for Europe (UNECE) to review its so-called "Gothenburg Protocol to Abate Acidification, Eutrophication and Ground-level Ozone". Nitrogen use efficiency (NUE) and N balance will be used as two key indicators in this international convention in order to assess the efficacy of measures to decrease nitrogen (N) losses while maintaining agricultural productivity [46]. Recently, Pires et al. [47] demonstrated that increase in NUE would lead to reduced N fertilization in cereal production as well as improve agronomic, economic and environmental benefits. Considering that increase in global fertilizer consumption is expected to reach 69 million tons in 2030, and the increased use of nitrogen (N) fertilizers is responsible for 67% of this amount. Commercial medicinal plant cultivation will certainly exacerbate this problem in the future. Therefore, it is important for countries to develop efficient policies and guidelines for use of N fertilization going forward.

10. Conclusion and recommendation

Nitrogen fertilization will be an important factor in commercial medicinal crop cultivation. In order to ensure sustainable commercial cultivation of medicinal plant, it is, therefore, necessary to develop efficient N fertilization management programmes as well protocols and policies. It is important that caution is exercised when implementing N fertilization in commercial farming of medicinal plants and the following important aspects should be addressed:

- Determine N requirements of each medicinal plant species and cultivar to ensure optimum quality and yield of medicinal materials while minimizing toxicity to plants, environment and consumers.
- Establish the best types (organic or inorganic) and source (salt, compost and manure) of N fertilizers, which will ensure optimum plant growth with reduced financial and environmental costs.
- Good knowledge of the physical and chemical properties of plant growth media is important because physical and chemical properties of soil vary geographically and this will certainly impact on cation exchange, porosity and organic matter which will in turn affect plant uptake of N. In hydroponics, substrates influence water retention and uptake of nutrients.
- Assess the cost of fertilizer inputs and selling price of medicinal produce in the short- and long-term.
- It is recommended that collaborative partnerships between research, training institutions and commercial farmers be established. Further studies that seek to develop optimized cultivation protocols and policies might be carried out in order to achieve high yield and high quality materials, and sustainable commercial cultivation of medicinal plant.
- Farmers should familiarize themselves with the relevant policies and regulatory frameworks.

Author details

Felix Nchu*, Yonela Matanzima and Charles P. Laubscher

*Address all correspondence to: felixnchu@gmail.com

Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville, Cape Town, South Africa

References

- [1] Balick, M.J. and Cox, P.A. 1997. Ethnobotanical research and traditional health care in developing countries. In: Bodeker, G., Bhat, K.K.S., Burley, J. and Vantomme, P. Medicinal Plants for Forest Combination and Healthcare. Food and Agriculture Organization of the United Nations. pp. 12-23. <http://www.fao.org/3/a-w7261e.pdf>
- [2] Motaleb, M. A. 2010. Approaches to conservation of medicinal plants and traditional knowledge: A focus on the Chittagong Hill Tracts. IUCN (International Union for Conservation of Nature), Bangladesh Country Office, Dhaka, Bangladesh, pp. 8-30. <https://www.iucn.org/sites/dev/files/import/downloads/medicinalplant11book.pdf>

- [3] Dold, A. P. and Cocks, M.L. 2002. The trade in medicinal plants in the Eastern Cape Province of South Africa. *South African Journal of Science* **98**, 589-597.
- [4] Wiersum, K.F., Dold, A.P., Husselman, M. and Cocks, M. 2006. Cultivation of medicinal plants a tool for biodiversity conservation and poverty alleviation in the Amathole region, South Africa. *Medicinal and Aromatic Plants* **3**, 43-57.
- [5] Mander, M. 1998. Marketing of Indigenous Medicinal Plants in South Africa: A Case Study in Kwazulu-Natal. Food and Agricultural Organization of the United Nations, Rome, Italy. pp. 1-12.
- [6] Van Wyk, B.-E., van Oudtshoorn, B. and Gericke, N. 2009. *Medicinal Plants of South Africa*. Briza Publications, Pretoria, South Africa. pp. 1-250
- [7] Bone, K. and Mills, S. 2013. Principles of herbal pharmacology. In: *Principles and Practice of Phytotherapy (Second Edition)*, edited by Kerry Bone and Simon Mills, Churchill Livingstone, Saint Louis. 2013. pp. 17-82, <http://dx.doi.org/10.1016/B978-0-443-06992-5.00002-5>
- [8] Kumar, A., Singh, S.P. and Bhakuni, R.S. 2005. Secondary metabolites of *Chrysanthemum* genus and their biological activities. *Current Science*, **89**, 1489-1502.
- [9] Vu, T.D., Tran, T.L.M., Biteau, F., Mignard, B., Fevre, J.P., Guckert, A. and Bourgaud, F. 2006. Improvement of secondary metabolites production in hydroponic cultures by mechanical and biological processes. *Proceedings of International Workshop on Biotechnology in Agriculture*, Nong Lam University Ho Chi Minh City October 20, 21 2006. pp. 195-200.
- [10] Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L. and Suzuki, A. 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany*, **105**(7), 1141-1157. doi: 10.1093/aob/mcq028.
- [11] Sobiecke, J.-F. 2014. The intersection of culture and science in South African traditional medicine. *Indo-Pacific Journal of Phenomenology*, **14**(1), 1-13.
- [12] Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., Paul, A. and Devasagayam, T. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of Clinical Biochemistry and Nutrition*, **40**(3), 163-173. <http://doi.org/10.3164/jcbl.40.163>.
- [13] Maroyi, A. 2013. Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. *Journal of Ethnobiology and Ethnomedicine*, **9**, 31. doi: 10.1186/1746-4269-9-31.
- [14] Shinwari, Z.K., Rehman, M., Watanabe, T. and Yoshikawa, Y. 2006. *Medicinal and Aromatic Plants of Pakistan (A Pictorial Guide)*. Kohat University of Science and Technology, Kohat, Pakistan, 492p.
- [15] Street, R.A. and Prinsloo, G. 2013. Commercially important medicinal plants of South Africa: a review. *Journal of Chemistry*, **2013**, 1-15. <http://dx.doi.org/10.1155/2013/205048>

- [16] Loundou, P.-M. and Watts, S. 2008. Medicinal Plant Trade and Opportunities for Sustainable Management in the Cape Peninsula, South Africa. Unpublished Thesis, University of Stellenbosch, South Africa. pp. 1-103.
- [17] Rashid, A.Z.M.M., Tunon, H., Khan, N.A. and Mukul, S.A. 2014. Commercial cultivation of medicinal plants in Northern Bangladesh. *European Journal of Environmental Sciences*, **4**, 60-68.
- [18] Schippmann, U., Cunningham, A.B., Leaman, D.J. 2002. Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues. Case Study No. 7. Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries, FAO, Rome, pp. 12-13. Visited 20th November 2014. <http://www.fao.org/docrep/005/y4586e/y4586e08.htm#TopOfPage>
- [19] Boczulak, S.A., Hawkins, B.J., and Roy, R. 2014. Temperature effects on nitrogen form uptake by seedling roots of three contrasting conifers. *Tree Physiology*, **34**, 513-523.
- [20] Johanna, B.M. 2007. Variation of Active Constituents in *Euclea nataliensis* Based on Seedling Stages, Seasons and Fertilizers. Unpublished MSc dissertation, University of Pretoria, South Africa. pp. 1-93.
- [21] White, A.G., Davies-Coleman, M.T. and Ripley, B.S. 2008. Measuring and optimizing umckalin concentrations in wild harvested and cultivated *Pelargonium sidoides* (Geraniaceae). *South African Journal of Botany*, **74**, 260-267.
- [22] Jehnson, M.H. 1999. Hydroponics worldwide. *Acta Horticulture*, **48**, 719-730
- [23] Hayden, A.L. 2006. Aeroponic and hydroponic systems for medicinal herb, rhizome and root crops. *HortiScience*, **41**, 536-538.
- [24] Koohakan, P., Ikeda, H., Jeanaksorn, T., Tojo, M., Kusakari, S., Okada, K. and Sato, S. 2004. Evaluation of the indigenous micro-organisms in soilless culture: occurrence and quantitative characteristics in different growing systems. *Scientia Horticulturae*, **101**, 179-188.
- [25] Gontier, E., Clement, A., Tran, T.L.M., Gravot, A., Lie'vere, K., Guckert, A. and Bourgaud, A. 2002. Hydroponic combined with natural or forced root permeabilization: a promising technique for plant secondary metabolite production. *Plant Science*, **163**, 723-732.
- [26] Canter, M., Adeline, D. and Teadora, P. 2007. Researches concerning rooting technology of *Pelargonium* genus. *Bulletin USAMV-CV*, **64**, 1-2.
- [27] Argyropoulou, K., Salahas, G., Hela, D. and Papasavvas, A. 2015. Impact of nitrogen deficiency on biomass production, morphological and biochemical characteristics of sweet basil (*Ocimum basilicum* L.) plants, cultivated aeroponically. *Agriculture & Food*, **3**, 32-42. ISSN: 1314-8591.
- [28] Abdolzadeh, A., Hosseinian, F., Aghdasi, M and Sadgipoor, H. 2006. Effects of nitrogen sources and levels on growth and alkaloid content of periwinkle. *Asian Journal of Plant Sciences*, **5**, 271-276.

- [29] Lou, Y. and Baldwin, I. 2004. Nitrogen supply influences herbivore-induced direct and indirect defences and transcriptional responses in *Nicotiana attenuata*. *Plant Physiology*, **135**, 496-506.
- [30] Le Bot, J., Benard, C., Robin, C., Bourgaud, F. and Adamowicz, S. 2009. The “trade-off” between synthesis of primary and secondary compounds in young tomato leaves is altered by nitrate nutrition: experimental evidence & model consistency. *Journal of Experimental Botany*, **60**, 4301-4314.
- [31] Yañez-Mansilla, E. Cartes, P., Reyes-Díaz, M., Ribera-Fonseca, A., Rengel, Z., Lobos, W. and, Alberdi, M. 2015. Leaf nitrogen thresholds ensuring high antioxidant features of *Vaccinium corymbosum* cultivars. *Journal of Soil Science and Plant Nutrition*, **15**(3), 574-586.
- [32] Bottoms, T.G. 2012. Nitrogen requirements and N status determination of lettuce. *HortScience*, **47**, 1768-1774.
- [33] Fontes, P.C.R., Braun, H., Busato, C. and Cecon, P.R. 2010. Economic optimum nitrogen fertilization rates and nitrogen fertilization rate effects on tuber characteristics of potato cultivars. *Potato Research*, **53**, 167-179. doi: 10.1007/s11540-010-9160-3
- [34] Farquharson, R.J., Chen, D., and Li, Y. 2010. What is the impact on farmer nitrogen fertilizer use of incorporating the effects of nitrous oxide emissions? 19th World Congress of Soil Science, Soil Solutions for a Changing World 1 – 6 August 2010, Brisbane, Australia.
- [35] Nyborg, M., Malhi, S.S. Mumy, G. Kryzanoski, L. Pennt, D.C. and Laverty, D.H. 2008. Economics of nitrogen fertilization of barley and rapeseed as influenced by nitrate-nitrogen level in soil. *Communications in Soil Science and Plant Analysis*, **30**, 589-598. <http://dx.doi.org/10.1080/00103629909370229>
- [36] Xego, S. Kambizi, L. and Nchu, F. 2016. Threatened medicinal plants of South Africa: case of the family hyacinthaceae. *African Journal of Traditional Complement Alternative Medicine*, **13**(3), 169-180.
- [37] Bargmann, C.J. 2005. Geology and wine in South Africa. *Geoscientist*, **15**, 4-8.
- [38] The Non-Affiliated Soil Analysis Work Committee. 1990. Handbook of Standard Soil Testing Methods for Advisory Purposes. Soil Science Society of South Africa, Pretoria.
- [39] Campbell, C. R. and Plank, C.O. 1998. Preparation of plant tissue for laboratory analysis. In: Kalra, Y.P. (ed.). Handbook of Reference Methods for Plant Analysis. CRC Press, Boca Raton, Fla, USA. pp. 37-49.
- [40] Miller, F. C.1992. Composting as a process based on the control of ecologically selective factors. In: Blaine-Metting, F. (ed.). Soil Microbial Ecology: Applications in Agriculture Environment Management, Marcel Dekker Inc., New York. p. 646.
- [41] Eloff, J.N. 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology*, **60**, 1-8.

- [42] Qiu, W., Wang, Z., Huang, C., Chen, B. and Yang, R. 2014. Nitrate accumulation in leafy vegetables and its relationship with water. *Journal of Soil Science and Plant Nutrition*, **14**(4), 761-768.
- [43] Walker, R. 1990. Nitrates, nitrites and N-nitrosocompounds, a review of the occurrence in food and diet and the toxicological implications. *Food Additives and Contaminants*, **7**, 717-768.
- [44] Stanton, L.T. 2001. Nitrate Poisoning. Colorado State University Extension, No.1.610.
- [45] Ju, X., Liu, X., Zhang, F. and Roelcke, M. 2004. Nitrogen fertilization, soil nitrate accumulation, and policy recommendations in several agricultural regions of China. *AMBIO: A Journal of the Human Environment*, **33**(6), 300-305.
- [46] Brentrup, F. and Palliere, C. 2010. Nitrogen Use Efficiency as an Agro-environmental Indicator. Accessed 19 June 2015. <http://www.oecd.org/tad/sustainable-agriculture/44810433.pdf>
- [47] Pires, M.V., da Cunha, D.A., de Matos Carlos, S. and Costa, M.H. 2015. Nitrogen-use efficiency, nitrous oxide emissions, and cereal production in Brazil: current trends and forecasts. *PLOS ONE*, **10**(8), e0135234. doi: 10.1371/journal.pone.0135234.