Chapter 11

Prospects of N Fertilization in Medicinal Plants Cultivation

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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), *Merwilla 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.

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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 NO_3–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 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 *Helichrysum cymosum* against *Fusarium oxysporum*	
	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

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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.

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