INTRODUCTION

Mankind has relied on Mother Nature for all its basic needs like food, shelter, medicine etc. since the start of recorded time. Medicinal plants (MP’s) have rendered their services to mankind for centuries forming the basis of traditional systems medicine because of their therapeutic value (Gurib-Fakim, 2006). These traditional systems of medicine are a result of the curative experience of several generations of practicing physicians of alternative medicine. As research on MP’s progresses, these plants continue to amaze us with new remedies through an admirable and ever increasing amount of modern drugs being isolated from them.

Of late an invigorated revival of MP’s from fringe to mainstream has occurred with increasing number of people turning to herbal remedies. The reasons for this resurgence and revivification include population explosion, scantiness, high cost and the associated side effects of synthetic drugs, evolution of resistant strains of highly communicable diseases, reappearance of vector borne diseases, better cultural acceptability of traditional medicine and better compatibility with body and lesser side effects of traditional medicine. Before proceeding any further it would be appropriate to write a few definitions of MP’s; what are MP’s? According to WHO (WHO, 1977) “MP’s are those plants which in one or more of their organs contains substances that can be used for therapeutic purposes or can act as precursors for the synthesis of useful drugs”. A more accepted definition provided by the agricultural and natural resource development board is “MP’s are those plants that are recognized by humans to have reliable prophylactic and lenitive properties against specific human ailments and thus play an essential role in health care”. Another definition of WHO (WHO, 2001) defines MP’s as plants, the herbal preparations, produced by extraction, purification, concentrations of components, of which, can be consumed for curative effects or can form a basis for herbal products.

Tracing the history of botanical medicine will be like tracing the history of humanity itself. The discovery of therapeutic potential of plants must have taken form instinctively from the use of plants as food. The ingestion of plant and plant products must have revealed some of these plant properties. MP’s formed the main source of health care compounds up until the nineteenth century. At present 80% of the people living in the developing countries like Pakistan, India and Sri Lanka rely on herbal medicine (Kamboj, 2000). In developed
countries the percentage, although lesser, is also picking up. For developed countries like Germany, USA, Australia and France the percentages are 40-50, 42, 48 and 49 per-cents respectively. According to WHO the use of herbal medicine in the world exceeds the conventional medicine by two-three folds (Evans, 1994).

The herbal economy is picking up in the whole world especially in the developed countries. Current herbal market stands at more than 100-150 billion US dollars per year (Kumari et al., 2011). European nations enjoy the largest market share of approximately 43% which amounts to nearly 43-65 billion US dollars per year. Table 1.1 presents an overview of the global nutrition product industry in the year 1999 including herbal medicine. Among the Asian countries China and Japan hold the largest share in the herbal trade industry. In India the herbal industry is worth ten billion US dollars (Kumari et al., 2011). The market for herbal products is estimated to be growing by 15-20% annually and according to a WHO estimate will be of the order of 5 Tn US dollars by the end of the year 2050 (Joshi et al., 2004). Figure 1.1 shows the growth trend of herbal industry from the year 1997-2000.

Table 1.1: The global nutrition products industry in 1999, including herbal and botanical products (in millions of US $)

<table>
<thead>
<tr>
<th>Country</th>
<th>Vitamins/minerals</th>
<th>Herbs/botanicals</th>
<th>Sports, meal replacement</th>
<th>Natural foods</th>
<th>Natural personal care</th>
<th>Functional foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>7070</td>
<td>4070</td>
<td>4320</td>
<td>9470</td>
<td>3590</td>
<td>16080</td>
</tr>
<tr>
<td>Europe</td>
<td>5670</td>
<td>6690</td>
<td>2510</td>
<td>8280</td>
<td>3660</td>
<td>15390</td>
</tr>
<tr>
<td>Japan</td>
<td>3200</td>
<td>2340</td>
<td>1280</td>
<td>2410</td>
<td>2090</td>
<td>11830</td>
</tr>
<tr>
<td>Canada</td>
<td>510</td>
<td>380</td>
<td>250</td>
<td>700</td>
<td>330</td>
<td>1500</td>
</tr>
<tr>
<td>Asia</td>
<td>1490</td>
<td>3170</td>
<td>970</td>
<td>710</td>
<td>880</td>
<td>1450</td>
</tr>
<tr>
<td>Latin America</td>
<td>690</td>
<td>260</td>
<td>250</td>
<td>460</td>
<td>250</td>
<td>360</td>
</tr>
<tr>
<td>Australia &amp; New Zealand</td>
<td>300</td>
<td>190</td>
<td>90</td>
<td>340</td>
<td>140</td>
<td>540</td>
</tr>
<tr>
<td>Eastern Europe &amp; Russia</td>
<td>350</td>
<td>220</td>
<td>250</td>
<td>180</td>
<td>40</td>
<td>269</td>
</tr>
<tr>
<td>Middle East</td>
<td>180</td>
<td>90</td>
<td>60</td>
<td>70</td>
<td>30</td>
<td>140</td>
</tr>
<tr>
<td>Africa</td>
<td>160</td>
<td>80</td>
<td>70</td>
<td>80</td>
<td>10</td>
<td>120</td>
</tr>
<tr>
<td>Total global</td>
<td>19 260</td>
<td>17 490</td>
<td>9 960</td>
<td>22 700</td>
<td>11 020</td>
<td>47 670</td>
</tr>
</tbody>
</table>

India occupies a central place in herbal medicine, the use of which dates back to 5000 years BC. Rigveda and Atharveda form the most ancient pieces of Indian literary on the use of herbal medicine. India is blessed with several traditional systems of medicine like Ayurveda, Yoga, Sidha, Unani and Homeopathy. India boasts of more than one fourth shares of worlds
known MP’s amounting to almost 30,000 plant species. About 90% of these grow naturally in the wild. India has a substantial market share in the trade of MP’s with a turnover of approximately one billion US dollars.

**Figure 1.1: Trends in the global herbal nutrition products industry, 1997-2000**

The entities of interest from these herbs are their active constituents also referred to as secondary metabolites (SM’s), the physiological responses of the human body towards which has rendered them to be used globally. These active constituents or SM’s are a large array of chemicals categorized into groups like alkaloids, terpenoids, lignons, flavonoids, glycosides and phenols etc. Terpenoids form the dominant group among them accounting to almost one third of the all known compounds. Each plant has its own set of specific SM’s which are mainly synthesized by these plants as defenses against herbivory and ecological variations (Wink, 1988). The SM’s are by-products of primary metabolism and their production is triggered by cell differentiation processes (Yeoman et al., 1982). According to world health organization 20-25 per-cent of modern medicine have their origins from plants first used traditionally. Several others are built on prototype of some SM’s (Balandrin et al., 1985). Most of the plant drugs are either these purified SM’s or they are partially synthesized analogues, such as atropine, aspirin, caffeine, ephedrine, digoxin, sienna, quinine,
theophylline, tubocuraine, warfarin, cough suppressants and the opioid narcotics. A lot of newly discovered drugs like artemesinin, taxol, vincristine and vinblastine also owe their origin to plants. The chart depicted in figure 1.2 shows all new chemical entities from 1981-2010 arranged by their source. The instant rise in the demand of these SM’s has put a lot of pressure on the natural populations of these plants. It is ironical that despite their vital importance, the MP’s are under heavy and ruthless exploitation. Most of these MP’s have slow rates of growth with low population densities spread in narrow geographic ranges (Nautiyal et al., 2002). Therefore the un-regulated exploitation has culminated into the breakdown of the balance between the availability of raw materials and growth of bulk demand which in turn has pushed some of these medicinal plants towards extinction (Jablonski, 2004).

**Figure 1.2: All new chemical entities from 1981-2010 arranged by their source**

* “B”: Biological; usually a large (>45 residues) peptide or protein either isolated from an organism/cell line or produced by biotechnological means in a surrogate host. “N”: Natural product. “ND”: Derived from a natural product and is usually a semi-synthetic modification. “NB”: Natural product “Botanical. “S”: Totally synthetic drug, often found by random screening/modification of an existing agent. “S*”: Made by total synthesis, but the pharmacophore is/was from a natural product. “V”: Vaccine. One subcategory is used. “NM”: Natural Product Mimic.

Considering the human health scenario at present it is expected that the demand for the SM’s will only rise in the future. This demand can only be managed in two ways.
1. Screening of high yielding plants having tolerance towards nutrient deficiencies and environmental stresses and their introduction into agriculture.

2. There is also a room for improving the plant SM’s which are present in the plants only in trace amounts. This can be tried in an environmentally friendly way by improving plant nitrogen use efficiency (NUE) through the incorporation of other nutrients or plant growth regulators having a direct influence on primary metabolism of these plants.

Increasing nitrogen (N) application rates have nourished the growing population and are expected to significantly chip in to nourish the ever growing human fleet through increased crop production (Tilman et al., 2002). But experiments are perpetually showing that the amount of N supplied to the crop is not fully recovered through yields, and this recovered N tends to decrease with increasing inputs of N (Cassman, 1999). Such high N input systems lead to diminished yields with high N inputs. Therefore meliorating N management in the croplands of the world is necessary for improving agricultural productivity and the wellbeing of human race (Compton et al., 2011; Foley et al., 2011; Galloway et al., 2008; Mueller et al., 2012; Tilman et al., 2002).

Efficiency of the uptake of N and its use is subject to the effective operation of processes associated with its absorption, assimilation, translocation and redistribution in the plant. These aspects though well studied in crop plants have not been examined in MP’s especially *Andrographis paniculata*. Several strategies have been put forward from time to time for improving NUE in plants. The amount of N available to the plants can be improved by following certain management strategies like the inclusion of other mineral nutrients or plant hormones as already discussed. Plant nutrition is of pivotal importance and the availability of mineral nutrients often defines plant growth and yield. The growth and development of plants are controlled through specific chemicals produced within the body of the plants i.e. hormones (Aguilo et al., 2005). Incorporation of some plant hormones have been shown to play a critical role in NUE (Iqbal et al., 2012; Nazar et al., 2011) and in the biosynthesis of plant SM’s (Jaleel et al., 2006).

*A. paniculata* (Burm.f.) Wall ex Nees (Acanthaceae) is an important herbaceous medicinal plant. Commonly referred to as Kalmegh and King of bitters, *A. paniculata* is a strong therapeutic pharmacophore with potential anti-inflammatory and anti-allergic properties. The
plant is prevalent in both the Ayurvedic and Unani systems of medicine. For several centuries the plant has been exploited in the treatment of fevers and upper respiratory tract infections. The plant is native to several Asian countries with a tropical and subtropical climate (Bhakuni et al., 1969; Chadha, 1976). Modern scientific evidence is in support of remedial properties of this plant towards several human ailments. The plant acts as an anti-bacterial, vermicidal, anti-typhoid, anti-acne, analgesic, hypoglycemic and anti-biotic, besides being a hepato-protectant and immunity enhancer (Saxena et al., 2000). More than hundred active principles have so far been reported from this plant including mainly diterpene lactones. However certain flavonoids, norrorids, polyphenols and stigmasterols have also been reported (Sareer et al., 2014; Xu et al., 2012). Whole plant is used for extraction of its active constituents; the bicyclic diterpene lactones, viz. andrographolide, neoandrographolide, 14-deoxyandrographolide and 14-deoxy-11, 12-didehydroandrographolide. Of these, andrographolide is by far the most biologically active constituent. The concentration of active principles is highest in the leaves.

Modern pharmacological studies have shown that andrographolide protects liver and gallbladder against toxicity induced by several agents. Neo-andrographolide is active against the malarial parasite Plasmodium (Misra et al., 1992) and is also hepato-protective against toxicity induced by CCL₄ (carbon tetrachloride) (Kapil et al., 1993). 14-deoxy andrographolide has been shown to possess a potent hypotensive effect in anaesthetized rats (Zhang et al., 1998). Pharmacological evidence is also present in support of anti-cancer and anti HIV properties of A. paniculata (Ajaya Kumar et al., 2004; Calabrese et al., 2000).

The quality of the drug to the industry is, however, inconsistent due to wide variation in environmental conditions at different locations, lack of proper cultivation packages, and also due to genetic variation of the material. The present study was undertaken with the objective of improve NUE, growth, herbage yield and andrographolide content in the selected accessions of A. paniculata. Following were the broad objectives of this work.
Chapter 1. 

Introduction

Objectives

1. To screen *A. paniculata* accessions for total andrographolide content, yield and its components.
2. To analyze the mineral-nutrition induced changes in nitrogen use efficiency and andrographolide content in selected genotypes.
3. To analyze the plant growth regulators induced changes in nitrogen use efficiency and andrographolide content in selected genotypes.
4. To establish the cultivation package of the selected genotypes of *A. paniculata* for enhanced biomass accumulation and yield of andrographolide.

To achieve the above objectives five different experiments were planned in three successive years. In the first year several accessions of *A. paniculata* were screened for growth, nitrate reductase activity and yield of active principles and two accessions one high yielding and one low yielding accession was screened out.

In the second year the two accessions were subjected to different treatments of mineral nutrients (Nitrogen and Sulfur) and their growth, physiological and biochemistry, N metabolism, secondary metabolite concentration and NUE was studied in relation to the applied mineral nutrients.

In the third year these two accessions were subjected to different treatments of plant growth regulators (24-Epipibrassinolide and Ethylene) and their growth, physiological and biochemical responses, N metabolism, secondary metabolite concentration and NUE was studied in relation to the applied mineral PGR’s.

Finally a detailed literature survey was done to find the standard and best responsive way of cultivation of the plant. The results were combined with the findings of the present research in order to develop a package for better cultivation of this plant. Detailed scheme of experimentation and the methodology employed therein is provided in the fourth coming section materials and methods.