

Chapter 1

Introduction

1.1. *Withania somnifera*: An overview

Withania somnifera (L.) Dunal is an important medicinal plant of family Solanaceae. It has been extensively used in Indian, Unani and African traditional medicine (Kulkarni *et al.*, 1996; Kumar and Choyal, 2012; Mishra *et al.*, 2000; Murthy *et al.*, 2008). Its generic name *Withania* commemorates the English paleo-botanist, Henry Thomas Maire Witham, with an orthographic variation of the last alphabet 'm' into 'n' with the addition of a commemorative termination 'ia' and the specific epithet *somnifera* is derived from two Latin words 'somnus' (meaning- sleep) and 'fero' (meaning- to bear) indicating towards its sleep inducing properties (Mir *et al.*, 2012). It is commonly called as Ashwagandha, Asgandh, Indian ginseng, Pevetti or Winter cherry. Its name Ashwagandha has been derived from two words 'ashva' (meaning- horse) and 'gandha' (meaning- smelling) because of resemblance of the smell of its roots to a sweating horse (Rajeswara Rao *et al.*, 2012). Due to its immense therapeutic potential, *W. somnifera* appears in World Health Organization (WHO) monographs on selected medicinal plants (Mirjalili *et al.*, 2009).

1.2. Origin and distribution

Withania somnifera is believed to be originated in Mediterranean region and Orientalis (Singh and Kumar, 1998) and it is widely distributed in dry arid soils of tropical and sub-tropical regions. The plant grows in wild condition in Southern Mediterranean region, Canary Islands, South and East Africa, Congo, Madagascar, Palestine Israel, Jordan, Egypt, Sudan Iran, Afghanistan, Baluchistan, India and Pakistan (Gifri, 1992; Kumar *et al.*, 2007; Rajeswara Rao *et al.*, 2012; Singh and Kumar, 1998; Udayakumar *et al.*, 2013; Wood, 1908) (Fig. 1.1a). In India, the plant grows well throughout the drier parts and in the sub-tropical and semi-temperate regions, including the states of Maharashtra, Madhya Pradesh, Gujarat, Rajasthan, Uttar Pradesh, Haryana and Punjab extending to Himachal Pradesh and Jammu and Kashmir (Fig. 1.1b), from plains to a height of 1,700 meters (Deb, 1980; Gupta, 1964; Mirjalili *et al.*, 2009; Nayar, 1964; Nigam, 1984; Verma *et al.*, 2012). It is cultivated mostly in Mansa, Neemuch and Jawad tehsils of Manduasur district of Madhya Pradesh, some parts of Kota district of Rajasthan, Andhra Pradesh and Uttar Pradesh (Kakaraparathi *et al.*, 2013; Kumar *et al.*, 2007, 2009a; Mir *et al.*, 2012; Mirjalili *et al.*, 2009; Nigam, 1984). The other cultivated species of *Withania* is *W. coagulans*, which is commonly called Vegetable rennet, is generally found in Afghanistan, India, Iran and Pakistan (Ahmad, 2007; Gilani *et al.*, 2009; Mirjalili *et al.*, 2009). In India, it is well distributed throughout the drier areas of Punjab and Rajasthan (Negi *et al.*, 2006; Panwar and Tarafdar, 2006).

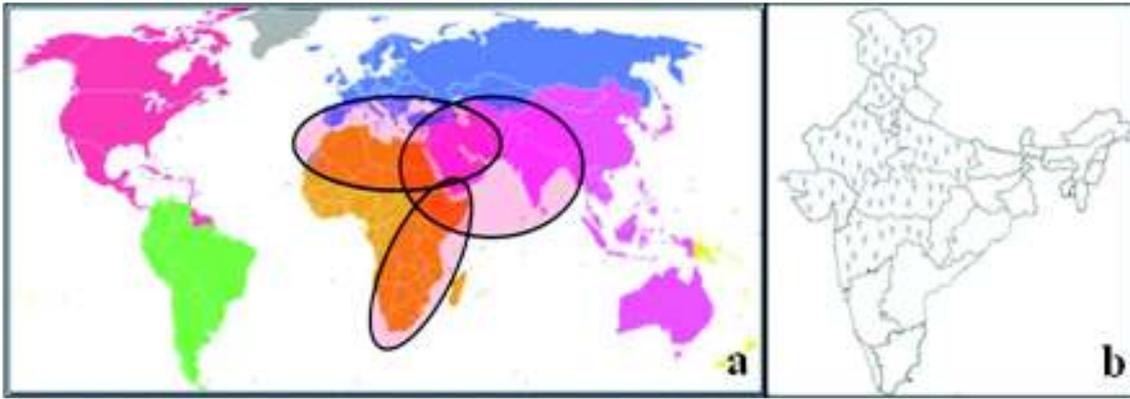


Fig. 1.1. Distribution of *Withania somnifera*. **(a)** World. **(b)** India. Circles indicate the areas where the plant grows in the world. Distribution on the maps was shown using Microsoft PowerPoint.

1.3. Botanical description and reproductive biology

Withania somnifera is an erect, dicotyledonous, evergreen, perennial under-shrub with branched, hairy-tomentose stem bearing broadly ovate, pubescent, petiolate leaves 5.0-8.5 × 3.0-5.0 cm (length × breadth) (Fig. 1.2a, b). Leaves are entire along the margins, acute to obtuse from the apex, dorsi-ventral with a prominent midrib (with broad abaxial hump) and its lamina is bilaterally symmetrical with abundant epidermal trichomes on its abaxial side. Leaves on their axil bear umbelate cyme cluster of pale green flowers (Fig. 1.2b). The flowers which appear throughout the year are sessile to sub-sessile, shortly pedicellate, pentamerous, hermaphrodite, yellow to dull green in colour and 4-6 mm in diameter (Fig. 1.3c-f). Calyx is gamosepalous, campanulate, green and sepals are five in number. Corolla is gamopetalous, campanulate, greenish yellow, lobes are 2.0-2.5 mm long, triangular and are tomentose to the outside. Anthers are five in number, sub-included and anther filaments are 1.0-2.5 mm long. Fruit (berry) is enclosed in persistent inflated calyx (Fig. 1.2g-l). Berries are globose, 5-8 mm broad, containing numerous, small, sub-pyriform to reniform, minutely reticulate-foveolate, yellowish-brown, smooth discoid seeds.

Floral development in *W. somnifera* has been extensively studied to understand its reproductive behaviour. Its flowers exhibit partial temporal dichogamy of protogynous type (Lattoo *et al.*, 2007) in which stigma attains receptivity about 48 h prior to anther dehiscence and it remains exerted beyond the reach of staminal cone (Fig. 1.2c, e). Normally the insect pollinators facilitate the cross pollination of receptive stigma. However, in case of non-receipt of cross-pollen due to absence of insect pollinators the autogamous fertilization is assured by upward movement of staminal cone by elongation of filaments, and keeps the staminal cone in close proximity to stigma (Fig. 1.2d, f). Hence, *W. somnifera* plants are of mixed mating

type (both cross and self-pollinating), that ensures high reproductive success (Lattoo *et al.*, 2007). Its pollens however are more fertile in winters (85-96%) in comparison to summers (65-75%) (Singh, 2009).



Fig. 1.2. (a) *Withania somnifera*: a high value medicinal plant. (b) Flowering twig with axillary flowers. (c) Flower of *W. somnifera* showing receptive stigma beyond the reach of staminal cone. (d) Flower of *W. somnifera* showing upward movement of staminal cone by elongation of filaments flower. (e-h) Transverse sections of flower, showing fruit development. (i-j) Stages of fruit development within inflated calyx (k) Ripened fruit enclosed in inflated calyx (l) Mature fruit (red berry). (m) Floral diagram and floral formula of *W. somnifera*. Photographs were taken using digital camera and stereo-microscope, and floral diagram was drawn using Microsoft PowerPoint.

1.4. Economic importance

Withania somnifera possess immense therapeutic potential and is used against a large number of ailments. It is known for its immuno-modulatory (Malik *et al.*, 2009; Rasool and Varalakshmi, 2006), anti-stress (Archana and Namasivayan, 1998), cardioprotective (Mohanty *et al.*, 2004), anti-aging (Singh *et al.*, 2008), antioxidant, anti-inflammatory (Mishra *et al.*, 2000) and anti-tumour properties (Devi, 1996). It was recently reported that *W. somnifera* leaf extracts selectively kill cancer cells (Widodo *et al.*, 2007). The medicinal values of *W. somnifera* are attributed to the presence of a wide array of secondary metabolites. The presence of important secondary metabolites such as alkaloids, flavanol glycosides, glycowithanolides, sterols, steroidal lactones and phenolics have been reported (Chatterjee *et al.*, 2010; Chaurasiya *et al.*, 2009, 2012; Ghosal *et al.*, 1988; Kandil *et al.*, 1994; Lockley *et al.*, 1974; Sangwan *et al.*, 2008; Xu *et al.*, 2011; Yu *et al.*, 1974). Alkaloids isolated from *W. somnifera* growing in wild include tropine, psudotropine, hygrine, 3-trigloyloxytropine, cushohygrine, choline, dl-isopelletierine, anaferine, anahygrine and withanosomine (Schwartzing *et al.*, 1963; Schröter *et al.* 1966).

In *W. somnifera*, leaves (Jayaprakasam *et al.*, 2003) and roots (Kumar *et al.*, 2011) are major plant parts used in herbal medicine. However, its bark, seeds (Kulmi and Tiwari, 2005) and fruits are rarely used (Abou-Douh, 2002; Bolleddula *et al.*, 2012). Recently, a total of 62 major and minor primary and secondary metabolites from leaves and 48 from roots have been identified, out of which 29 are common to both (Chatterjee *et al.*, 2010). However, the distribution of secondary metabolites varies significantly with respect to different organs. Withaferin A and withanone are major metabolites present in leaves, whereas withanolide A is major metabolite found in roots (Chatterjee *et al.*, 2010). Among various secondary metabolites, the clinical roles of withaferin A and withanone are well established (Chaurasiya *et al.*, 2009; Grover *et al.*, 2012; Widodo *et al.*, 2007, 2010; Yang *et al.*, 2007). Their major site of synthesis is believed to be leaves.

1.5. Genetic and chemotypic variations

The genus *Withania* includes more than 23 species of which two species, *W. somnifera* and *W. coagulans* are found in India (Kumar *et al.*, 2012). The survey conducted on various geographical locations revealed the existence of distinct morphological variations in *W. somnifera* (Kumar *et al.*, 2007). The variability was observed in plant height (37.78-132.91 cm), number of branches per plant (2.33-8.00), number of seeds per berry (21.33-

41.08), root length (11.27-31.87 cm), root diameter (0.86-2.46 cm) and root yield (3.72-24.70 g). These morphological variations were later correlated with genetic variations. Genetic marker studies were conducted to access the genetic variability among different accessions collected from various geographical regions. Eighteen RAPD (Random Amplified Polymorphic DNA) primers and six AFLP (Amplified Fragment Length Polymorphism) primer combinations were used to study the genetic diversity of 23 accessions showed 37.82 and 43.94% polymorphism, respectively. AFLP revealed higher levels of polymorphism as compared to the RAPD. The data clearly indicated the existence of two distinct clusters. A clear distinction of cultivated and wild accessions was observed (Mir *et al.*, 2011). Further, this data to a large extent correlates with the morphological distinctness of different accessions. The genetic variability was also pronounced among the accessions collected from same geographical region. The analysis of accessions collected from different parts of Tamil Nadu, upon analysis with RAPD primers showed a very high degree of polymorphism (83.78%) (Dharmar and De Britto, 2011). In a comparative study, SAMPL (Selectively Amplified Microsatellite Polymorphic Loci) assay and AFLP were used to assess the levels of genetic diversity in different *W. somnifera* genotypes. SAMPL assay revealed higher levels of polymorphism as compared to AFLP. The use of SAMPL and cluster analysis suggested higher level of polymorphism among Kashmiri and Nagori genotypes. The divergence of these two genotypes based on their polymorphism clearly indicates a distinct grouping of Kashmiri and Nagori genotypes (Negi *et al.*, 2006). Study conducted using 12 different genotypes and 22 Inter Simple Sequence Repeat (ISSR) markers also suggested a wide variation (0.02-0.8333) among selected genotypes of *W. somnifera* (Bamhania *et al.*, 2013). An attempt has also been made to establish a link between the genetic variability and chemotypic variability. The analysis of different accessions using AFLP and subsequent analysis of the major active components suggested a high degree of correlation (Dhar *et al.*, 2006).

Based on their composition of major active components, *W. somnifera* plants have been classified into various chemotypes. Three different chemotypes of *W. somnifera* were recorded in Israel (Abraham *et al.*, 1968, 1975) and one each was recorded from South Africa and India (Kumar *et al.*, 2007). However, there are evidences for the presence of more than one chemotypes in India (Kumar *et al.*, 2007). The major steroidal lactones of chemotype I, II and III from Israel were withaferin A, withanolide D and withanolide E, respectively (Abraham *et al.*, 1968, 1975). Whereas, Indian chemotype is rich in withanone and withaferin

A (Anjaneyulu and Rao, 1997; Dhalla *et al.*, 1961; Kirson *et al.*, 1971). The main withanolides found in South African chemotype are withaferin A and withaferin D (Kirson *et al.*, 1970). Some new withanolides have also been characterized from the hybrids of Israel with Indian chemotypes (Bessalle *et al.*, 1987; Nittala and Lavie, 1981) and Israel and South African chemotypes (Eastwood *et al.*, 1980).

1.6. Rationale and objectives of the thesis

In the growing realization of large disadvantages of industrial chemicals and food, there is a global shift of preference towards natural chemicals and herbs for medicines and health supplements. World Health Organization (WHO) estimates that more than 80% of population in developing countries still relies primarily on traditional remedies such as herbs for their medicines (Haq, 2004). Moreover, approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from plant source. With such world-wide shift in consumer choice and preferences for herbs, there has been an intensive effort towards propagation of medicinal plants using rational scientific approach.

The infestation of various pests and pathogens is a major threat in cultivation of plants. It is realised that diseases have caused major yield losses and have impacted the well-being of humans worldwide (Agrios, 2005). There are also many reports on the bio-deterioration of the nutritive content of cereals, pulses, oil seeds, vegetables and fruits due to diseases (Amusa *et al.*, 2003; Bilgrami *et al.*, 1979; Singh *et al.*, 1990). Hence, it is imperative to identify these phyto-pathogens, study the disease establishment process and the post-infectious changes associated with it. Addition of knowledge in these aspects will certainly pave the way for developing suitable disease management strategies for a particular crop. *W. somnifera*, commonly known as Indian ginseng is an important medicinal plant that has been used in Ayurvedic and indigenous medicine for over 3,000 years (Mirjalili *et al.*, 2009). The important medicinal properties of this plant are attributed to presence of wide array of pharmaceutically important secondary metabolites. However, *W. somnifera* is prone to several diseases and pests both under wild and cultivated conditions (Sharma and Trivedi, 2010). It is attacked by several fungal pathogens (Mahrshi, 1986; Maiti *et al.*, 2007; Verma *et al.*, 2008), viruses (Pathak and Raychoudhuri, 1967), phytoplasmas (Khan *et al.*, 2006; Samad *et al.*, 2006), insects (Kumar *et al.*, 2009a, 2009b, 2009c; Nagraj and Reddy, 1985; Srivastava and Saxena, 1976), and nematodes (Sharma and Pandey, 2009). Further, in a study conducted in Saudi Arabia, various microorganisms including bacteria, actinomycetes and

fungi were recovered from the leaves of *W. somnifera*, collected from two different altitudinal ranges (Alwadi and Baka, 2001).

Quality control is an important issue in the cultivation of medicinal plants. The major concern of attack of pathogens is not only the deterioration of the active pharmaceutically important constituents of medicinal plant but also the associated probable consequence of pathogen secreted toxin in the host tissue. As per WHO guidelines on ‘Good Manufacturing Practices (GMP) for herbal medicines’ (WHO, 2007), it is not desirable to have either microbiological/fungal or myco-toxin/toxicant contamination in herbal products (Guideline number 15.4 and 15.9). Hence, to meet the international guidelines on quality assurance, it is imperative to develop a detection system against the specific pathogen affecting the host.

Keeping in view the above facts, the present study was designed with the following objectives:

1. Isolation, identification and characterization of fungal pathogen associated with leaf spot disease of *W. somnifera* (L.) Dunal.
2. Study of pathogenesis and post inflectional changes associated with disease.
3. Toxicity evaluation of diseased leaves of *W. somnifera* and development of a molecular detection system for leaf spot pathogen.