

Sem studies of adhatoda vasica: An endangered traditional medicinal plant of multifarious uses

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Abstract

The plant *Adhatoda vasica* is of immense medicinal importance. In the present study, seed morphology has been investigated scanning electron microscopy has been performed. Seeds showed variation in seed shape and size. Seed coat in *Adhatoda vasica* is essentially a structure formed by integument of ovule. The seed is unitegmic. A large portion of the integument is consumed by the developing endosperm and only the epidermis persists in mature seed. The epidermal cells of the seed coat form small rounded or elongated knob like structures on the surface of seed. They are made of very thick walled macrosclereid cells and have brown pigmentation. In surface view they look polygonal. They showed a pattern of smooth ridges and grooves below which are present stretched parenchymatous cells. SEM Studies show a pattern of tubercled or verrucose formations in surface view. Micropyle and hilum are prominently visible in the electron micrograph. Seed morphological characters are significant in identification of a particular species. The microphotographs are obtained with the help of scanning electron microscope (SEM)

Key Words: Acanthaceae, Scanning electron microscopy, Unitegmic.

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INTRODUCTION

Adhatoda vasica Nees syn. *Adhatoda zeylanica* Medik, syn. *Justicia Adhatoda* Linn belongs to family Acanthaceae is a well-known plant drug in Ayurvedic and Unani medicine¹⁸. It is a gregarious species of multipurpose use. It is distributed throughout India up to an altitude of 1300 m in the North West sub Himalayan tracts. Commonly cultivated as a hedge plant, it often grows wild near human inhabitations. It is found in dry and waste places and thrives well where other vegetation fails to grow. It may be due to its immunity from browsing by goats and other animals probably due to its fetid scent. Also elsewhere, it has almost gone as an

escape to become naturalized. The plant has been used in indigenous system of medicine in India for more than 2000 years¹ *Adhatoda vasica* Nees, (*A. vasica*), an evergreen local medicinal plant, is commonly employed for the treatment of cold, cough, asthma and tuberculosis, etc⁶. The stem, leaf, flower, fruit and seeds were used traditionally as a folk medicine¹⁹. It is a small evergreen, sub herbaceous bush. The leaves vary from 10-15 cms in length and are about 4cms broad. They are opposite, entire, minutely pubescent, broadly lanceolate and shortly petiolate tapering towards both apex and base. The inflorescence is dense, short pedunculate, bracteates with long bracts and spike terminal. The corolla is large and white with lower lip streaked purple or yellow. The fruit is a 4 seeded, non-fleshy, dehiscent, capsule which is longitudinally channeled. The seeds are globular, non endospermic, borne on minute hook like out growths, called "retinacula". It is also known for its antiarthritic, antiseptic, antimicrobial, expectorant, sedative and antituberculosis properties⁵. Chemical compounds found in leaves and roots of this plant includes essential oils, fats, resins, sugar, gum, amino acids, proteins and vitamin C etc⁷. Calorific value of seeds of *Adhatoda vasica* have been studied¹². The plant has been included in the WHO

manual The Use of Traditional Medicine in Primary Health Care²⁰ which aims to profit health workers in South-East Asia to keep them, informed of the therapeutic utility of their surrounding flora. Phenology of *Adhatoda vasica* has been studied¹³. Extracts of *Adhatoda vasica* are widely used in pharmaceuticals and traditional systems of medicines for a number of ailments^{4,14} studied the allopathic activity of *Adhatoda vasica*. Observations in many plant groups have shown that seed morphological characters are rather conservative which makes them taxonomically important. As study of seed structure of the genus *Adhatoda vasica* has been neglected by previous workers, attention has been paid to the seed structure of *Adhatoda vasica*. Seed morphology has provided useful characters for the analysis of taxonomic relationship in a wide variety of plant families. Surface study of seeds is useful in taxonomic discussion. In their interesting reviews^{3,17} have discussed systematic application of SEM studies of foliar epidermal characters in flowering plants.⁹ also stated that the micro structural properties of seed surface can be useful for delimiting taxa at various levels. In above context SEM studies will help in identifying and documenting authentic samples. The present study of *Adhatoda vasica* was undertaken to study the seed shape, size and its seed coat pattern.

MATERIALS AND METHODS

For the present study, area in and around Bhopal MP India was thoroughly surveyed for recording occurrence of *Adhatoda vasica* and selection of study sites where it was growing in wild conditions. Five sites were finally selected based on the consideration that they represented all types of areas where *Adhatoda vasica* grew naturally. The five sites so selected are:

Site 1: Motilal Vigyan Mahavidyalaya Campus, Bhopal MP India

Site 2: Lake view area along upper lake, Bhopal MP India

Site 3: Islamnagar Fort, Islamnagar. Bhopal MP India

Site 4: Raisen Fort, Raisen MP India

Site 5: Vidisha along state highway about 2 k.m. towards Sanchi. MP. India

Due to its typical fruit dehiscence and seed dispersal mechanism it was difficult to collect seed in large

OBSERVATIONS AND RESULTS

amounts. Hence, at each site 25 bushes were selected randomly and on them 100 inflorescence (4 per bush) were bagged using muslin cloth. Young buds and unopened flowers were removed from the inflorescence before bagging to ensure uniformity in seed size. The seed lots so collected were numbered and stored in bulk. From it working samples were taken for various studies to work upon for biochemical investigations, working samples were taken from the seed lot. The working sample is a representative sample suitable for use in which the probability of a constituent present is determined by its level of occurrence in the seed lot. This sampling was done by the soil type divider each working sample constituted of 25 seeds and three replicate of such sample were used for each treatment/investigation. Initiation of fruit setting was observed and mid of fruit setting with 50% fruit set is referred as peak of fruit setting. The percentage of fruit setting was determined by marking 50 spikes in each site and counting the number of flowers present and involved in fruit setting. For the study of dispersal of fruits/seeds observations were made during peak season of fruit maturation. Subsequently emergence of seedling in areas away from the shrubs was also studied to record the effectiveness of dispersal mechanism. Three samples, each having 25 randomly selected fruits from each site were examined for seed setting, viz number of seeds per fruit, seed abortion frequency, and variation in the incidence of seed setting. Random samples of *Adhatoda vasica* seeds were collected from selected sites where *Adhatoda vasica* grew naturally. Matured seeds were separated for investigation. The dry seeds were cleaned and examined by light microscope (LM) to study the shape and color of seed. For SEM investigations, the seeds were dried and fixed to specimen stubs with an adhesive and placed on the revolving discs of Joel fine coat ion sputter), where each seed was uniformly coated with 20-30 nm thick gold-palladium. These specimen stubs were then fixed to the specimen holder of Scanning Electron Microscope (JEOL Model, JSM 5600) maintained at accelerating potential voltage of 15 KV and photomicrographs were taken at different magnifications. The terms used for describing the seed coat patterns have been adopted according to^{16,10}



Figure 1a:



Figure 1b:



Figure 2a:

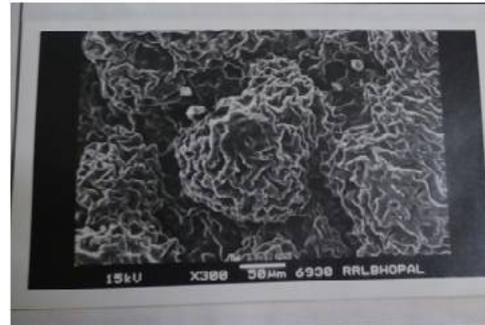


Figure 2b:

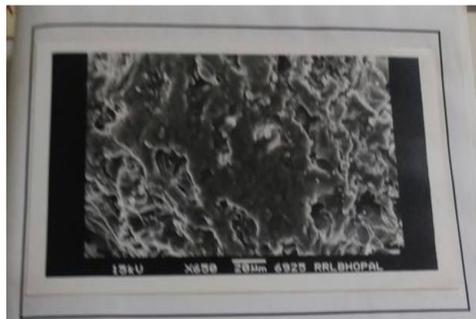


Figure 2c:

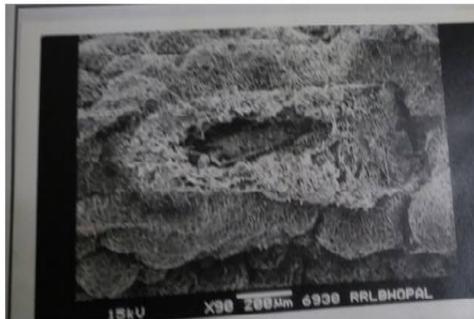


Figure 2d:

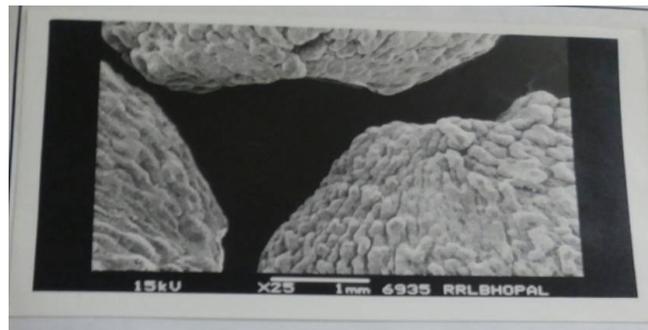


Figure 2e:

Figure 1a: Seed coat V.S showing Epidermal (40x 10 magnifications) and Hypodermal structure.

Figure 1b: Seed coat V.S showing Epidermal and Hypodermal structure (100 x10 magnification)

Figure 2a: SEM view of seed coat surface of *Adhatoda vasica* showing ridges and furrows (Verrucose tubercles) (100X magnification).

Figure 2b: SEM view of seed coat surface of *Adhatoda vasica* showing ridges and furrows (Verrucose tubercles) (300x magnification)

Figure 2c: SEM view of seed coat surface of *Adhatoda vasica* showing further details (650x magnification)

Figure 2d: SEM photograph showing micropyle and hilum region of *Adhatoda vasica* seeds magnification 90x.

Figure 2e: SEM photograph of seeds showing apical notch in the seed, 25x magnification.

The plant *Adhatoda vasica* is known as “Malabar nut” in English which is a misnomer. Its genus name is derived from a south Indian language (Tamil), meaning –plant not touched by goat, for “Aadu” means goat and “toda” means not touched in Tamil. Seeds of *adusa* are non endospermic, suborbicular, unitegmic, compressed, rugose, glabrous and tubercled (Fig-2). The colour of seed varies from dark brown to comparatively lighter shades of brown. Testa is tuberculate having distinct rounded knob like ridges and furrows. Hairs and scales are absent. There is a notch in the seed showing the attachment to funiculus (Hilum). The fruit is small, clavate and capsule containing four globular seed. Maximum length and breadth of seed is 4.4x3.6 mm. As the ovule develops into seed, the integuments mature into seed coats with very few exceptions; the ovule has either two integuments or just one. During their transformation into seed coats the integuments undergo significant histological changes. The seed coat in *Adhatoda vasica* is essentially a structure formed by integument of ovule. The seed is unitegmic. A large portion of the the integument is consumed by the developing endosperm and only the epidermis persists in mature seed. The epidermal cells of the seed coat form small rounded or elongated knob like structures on the surface of seed. They are made of very thick walled macroscleriedic cells and have brown pigmentation. In surface view they look polygonal. They showed a pattern of smooth ridges and grooves below which are present stretched parenchymatous cells. (Fig-1a,b). SEM studies show a pattern of tubercled or verrucose formations in surface view. Primary sculpture appears to be rugose reticulate having irregular cell walls. The anticlinal walls are depressed; therefore furrows are formed between the cells. The periclinal walls are convex and secondary sculpture appears to be rugose type. Cell shape is irregular. The surface appears to be an irregular network of string. Micropyle and hilum are prominently visible in electron micrograph. (Fig-2 a, b, c, d, e, f).

CONCLUSIONS

Seed morphology studies provide a number of characters potentially useful for species identification and phylogenetic inference^{2,8,15}. The seeds of *Adhatoda vasica* showed a variation in size and shape. SEM studies of *Adhatoda vasica* seed would be helpful for the identification of the species. Moreover, these results could be used to set new standards.

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