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Phenology and Reproductive Success of *Nicotiana pumbaginifolia* Vivane: A wild Tobacco

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Abstract: The present study on phenology and reproductive biology of *Nicotiana plumbaginifolia* Vivane. was undertaken. It produces flower throughout the year and maximum flowering recorded in the month of February however minimum flowering was recorded in the month of April. The flowers are complete, actinomorphic with a long narrow floral tube of about 3-4.5 cm long and 1.5-2 cm wide. The flowers open at 7:00 am to 9:00 am in the morning while in evening the flower opens at about 5:30 pm to 6:00 pm.One flower produces an average 5,36,000 pollen grains. The higher pollen viability (85%) was recorded by Alexander's stain while lower pollen viability (65%) was seen using 2,3,5-triphenyltetrazolioum chloride (TTC). The highest in vitro pollen germination in Brewbaker and Kwack's medium is (53.45%) with longest pollen tube 415 um however lowest pollen germination (46.40%) is recorded in 15% sucrose solution. The flowers attracted the vide variety of insects for the pollination but bees and hawk moth were most significant pollinators. The fruit-set percentage is calculated as 45%.

Index Terms-Flowering Phenology, floral biology, Pollination biology.

I-INTRODUCTION.

Reproduction is not only the most important and and a relatively fragile step in the life cycle of plants but also the core of their evolutionary process. Numerous records exist on the reproductive biology of wild

plants. Reproductive biology mainly focuses on flowering phenology, floral biology, pollen-pollinator interaction, pollen-pistil interaction, breeding system and gene flow through pollens and seeds. According to Shivanna (2003) pollen-pistil interaction is unique to flowering plants and it covers all sequential events those take place in the pistil starting from pollination until pollen tube entry into the embryosac. The environment in which an organism live affects its reproductive success of a plant (Sedgley and Griffin 1989). Environment exerts considerable influence on flowering, pollen fertility, in vitro pollen germinationand fruit setting in plants (Shivanna 2003). Numerous studies on reproductive biology of plants have been done like Kaul *et al.,* (2005) on *Withania somnifera,* Saha and Datta(2017) on *Solanum sisymbrifolium,* Singh *et al.,* (2010) on *Evolvulus alsinoides* and Jan Kova *et al.,* (2014) on *Tribulus terrestirs*. Saha and Datta(2014) worked done on reproductive biology of *Solanum viarum* Dunal in Northeast India.

Nicotiana plumbaginifolia Vivane (Fam. Solanaceae) is an erect annual plant up to 1m tall. It belongs to family Solanaceae which is generally known as night shade family, flowering annual herb of India It occurs on wastelands near water and is also found along river bank, railway tracks, roadsides and in cultivated fields (Sudhakar Reddy *et al.*,2018). It is a large family having two thousand and three hundered species, almost half of belongs to single genus *Solanum which includes small* trees, herbs and shrubs (Nasir, 1985). It is used in the treatment of cuts, wounds, toothache, rheumatic swelling in the traditional system of medicine (Dangwal *et al* ., 2010).

The objective of the prsent investigation is to understand the flowering phenology, floral morphology, floral biology, anthesis, pollen production, pollen viability, in vitro pollen germination and pollination biology. The detail insights on its reproductive biology of *N.Plumbaginifolia* will be helpful for the proper conservation, management, antimicrobial evaluation and genetic improvement.

II-MATERIALS AND METHODS

The present study was carried out during 2009-2010 in the climatic condition of Agra in North India. Floral phenology was observed in different plants were tagged before the initiation of flowering. These selected plants were followed daily and the number of open flowers was recorded. Twenty five flowers were sampled to record floral morphology. The plant produces bisexual flowers and on this basis flower ratio was calculated from daily anthesis observations. SEM (Scaning Electron Microscopy) was used to study the morphology of anther and pistil. Samples were fixed in 3 % glutarledehyde in 0.1 M phosphate buffer at Ph 6.8. the samples were dried with Co_2 at 1000lb per inch. The samples were mounted with gold 20mm in a SCD 0.2 sputter coating unit (Polaron equipment Ltd, Walford,England). Observations and photographs were taken using a Philips EM 501 SEM at AIIMS Delhi. The number of pollen grains per anther determined from 25 flowers by method used by cruden (1997). The pollen size was measured with an ocular micrometer under light microscope following the procedure of Mckone and Webb(1988). The

number of pollen grains divided by the number of ovules per flower yielded the pollen ovule ratio. . Pollen viability was tested using Alexander's (1980) staining technique and 1% TTC test. *In vivo* pollen germination was checked by method of Shivanna and Rangaswamy (1992). Pollination mechanism was checked by geitonogamy and xenogamy through hand pollination. the foregoing behavior of different insects was studied and the pollen efficiency was checked by observing the pollen load on different body parts of the insect under microscope, according to the procedure given by Kearn,s and Inouye (1993). The number of fruits formed on an inflorescence was recorded by randomly counting fruit formation per inflorescence.

III-RESULT AND DISCUSSION

Nicotiana plumbaginifolia Viv is an erect, branched perennial herb belonging to family solanaceae and commonly known as Jangali Tambakku. Leaves are radical and cauline, sessile 9x3.5cm in size, elliptic-oblong, wavy, cuneate to decurrent. Panicles up to 15 cm long. Pedicle up to 10mm long, glandular-pubescent. A phenological study was carried out on 25 plants at selected sit in Agra over a period of three year. Monthly observations regarding the following stage were made for individual plants: sprouting, leaf shedding, leaf renewal, bud initiation, flowering, fruiting and seed dispersal. It is a wild perennial herb grows up to height 1 meter. The leaf shedding and leaf renewal occurs in the month of January to April. However flush of new leaves appears in the month of August and continue to November. The maximum leaf shedding was recorded in the month of March to May and minimum leaf shedding was recorded in the month of June to July. The maximum leaf renewal occurs in the month of November to January however the minimum leaf renewal occurs in the month of March to April. The new bud initiation was recorded in the month of December where as maximum flowering observed in the month of February and minimum flowering was recorded in the month of April. The fruit formation starts in the month of March to April and fruits were matured in the month of April. The complete seed dispersal was recorded in the month of May.

Flowers are 2.0 cm in size; consist of calyx (9 mm) green in color and corolla (4.0 cm) yellowish in color. Stamens have 0.8cm long anther with 1.1cm long filament. They are five in number polyandrous, epipetalous, filament inserted near the base of floral tube. Anthers are tetrasprangiate dehiscing by apical pores and pollen grains are tetracolpate, prolate, psilate, tectate with long axis. The pistil is (3.9cm) long bicarpellary and ovary (0.3) is superior with a axile placentation. The style is filiform and stigma (0.2cm) is solid and bifid. The number of pollen grains per anther is 3700 while number of pollen grians per flower 18500. The number of ovules per flower 70. The pollen-ovule ration is calculated as 26.40:1. Coelho *et al* .,(2017) have reported the facts of reproductive biology of *Solanum melissarum* (solanaceae) and they updated its floral morphology and pollination mechanism. Anderson *et al.*,(2006) also studied the floral morphology of *Withania aristata* (Solanaceae). They found long floral tube of flower. Similar observation has been investigated by Andrea *et al.*, (2008) in *Solanum corolinense.*

SEM studies indicate that the pollen grains are subolate in shape with 35-45 um in diameter. The pollen grains are tricolpate, aperturate with reticulate exine sculpturing. The pistil is long, wet and shows the presence of small and loosely arranged paillae on the stigmatic surface. A considerable amount of pollen grains on stigmatic surface is observed. SEM photomicrographs of T.S and L.S of ovary shows that ovary is bilocular with numerous ovules. Flowers open early in the morning at 7:00am to 12:30 pm however some flowers open at evening at about 4:00 pm and remain close to 7:00pm (Table-1). There are large number of anther was dehisced at arround 11:00-12:00 am. The time of stigma receptivity was recorded in between 1:00 to 2:00 pm. Flowering and anthesis time may depends upon physical, physiological and biochemical factors of a plant as well as on climate conditions (Hamilton 1959, Reis & Kostic 1976). Douglas and Freyre (2010) also suggested floral development, stigma receptivity in some species of *Nolana* like *N. adansonii, N.laxa, N.elgana and N. humifera.* Kaczoroneski *et al.,* (2005) supported the findings of the present author. The present observation revealed higher pollen ovule ratio in *N.plumbaginifolia*. Sarma et al., (2007) found higher pollen ovule ratio in *Volvuopsis nummularium.*

The highest in vitro pollen germination in Brewbaker and Kwack's medium is (53.45%) with logest pollen tube 415 um however lowest pollen germination (46.40%) is recorded in 15% sucrose solution. The pollen viability as tested by Alexanders stain test and 1% TTC test, the pollen grains showed maximum viability in Alexander stain (80%%) however the minimum pollen viability is recorded in 1% TTC (75%) .The higher pollen viability was seen in Alexander's stain as compared by other pollen viability test. The pollen grain was considered vaible if it turned red in TTC and violet-purple in MTT (Sheffield *et al .,* (2005). Kaur *et al.,* (2011) reported 71% pollen viability in *J.curcas* using TTC technique. However or reultss showed that staining with TTC showed highest pollen viability. Pollen viability and the efficiency of pollen transfer partially determine the reproductive success of a species.

Flowers are self as well as cross-pollinated as confirmed by various hand pollination experiments. They were visited by different floral visitors namely butterflies (*Colias earytheme), honey bee (Apis dorsata, Apis cerna indica), black ant (Camponotus compestris), Bettle (Coccinella punctata), hercules ant (Camponotus herculeanus) Hawk moth (….)and chinch bug (Blissus leucopterus)*. They visit flowers in between 7:00 to 9 :00 am. It is interesting to note that all of them are not pollen carriers but honey bees, butterflies and bug are truly pollen carriers as proved by the presence of pollen load on their body parts is distictly observed on the lower side of abdomen. A large number of pollinators visit the flower of *N.plumbaginifolia* but the most efficient pollinatore were honey bees and hawk moth. Clivati *et al*., (2014) have studeid the pollination biology of *Utricularia reniformis* and they reported that bee as a efficeint pollinator. Anderson *et al.,* (2015) reported that the flowers of Solanum vespertilio (solanaceae) were buzz pollinated by large bodied native bees. *Nicotiana plumbaginifolia*, an annual herb is rich in polumethoxyflavones that possess significant analgesic and anxiolytic activities as confirmed by Shajib *et*

al., (2018). Ajaib *et al*.,(2016) suggested that *Nicotiana plumbaginifolia : a rich antimicrobial as well as antioxidant source.* Plants are important source of active natural products.

The fruit set percentage is calculated as (45%). The fruit is capsule, fleshy and oval or spherical in shape. The unripe fruit is green in color and it turns into brown or black at the time of maturity. It clear from the present observation that the average number of fruit\inflorescence is…the seed set percentage is calculated as (85%). The average number of seeds per fruit is 879. The seeds are round, subglobose to angular, minutely rugosereticulate, brown in color and the average size of seed is 1mm long. The weight of hundred seeds is measured as 0.009 gm.

Thus, on the basis of present observation and discussion it is clear that *Nicotiana plumbaginifolia* is a cross as well as self-pollinated medicinal herb and exhibits xenogamy as well as geitanogamy. The present study suggest that the population of this plant is slightly decreasing due to human exploitation such as urbanization, expansion of road network and low rainfall in the Agra region and require better strategies for propagation with the need of *ex-situ* and *in-situ* conservation.

Table 1. Floral biology of *Nicotiana pumbaginifolia*

Parameters	**Observations**
Flowering period	Throughout the year
Flower shape	Regular
Flower colour	Pale green to purplish
Odor	Present
Nectar	Absent
No. of Stamens	5
Flower open time	7:00-9:00am at morning while 4:00pm at evening
Number of pollen per flower	5,36,000
Anther dehiscence time	Just after flower opening
Mode of anther dehiscence	By longitudinal slits
Stigma	Simple, wet

Style	Long, terminal
Ovary	Superior
No. of ovules per flower	Numerous

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