

STUDIES ON REPRODUCTIVE ECOLOGY OF *Strychnos nux-vomica* L.(Loganiaceae)

By
ALOK MOHANTY
ADMN.NO- 03NRM/16



Department of Natural Resources Management
COLLEGE OF FORESTRY
ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
BHUBANESWAR-751003
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**A
THESIS SUBMITTED TO THE ODISHA UNIVERSITY OF AGRICULTURE AND
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By

Alok Mohanty

Adm. No. 03NRM/16



**DEPARTMENT OF NATURAL RESOURCES MANAGEMENT
COLLEGE OF FORESTRY ODISHA UNIVERSITY OF
AGRICULTURE AND TECHNOLOGY
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**ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
DEPARTMENT OF NATURAL RESOURCES MANAGEMENT
COLLEGE OF FORESTRY
BHUBANESWAR**

Mr Madhab Chandra Behera
Assistant Professor,
Natural Resources Management,
College of Forestry,
OUAT Bhubaneswar- 751003

Bhubaneswar
Date:

CERTIFICATE-I

This is to certify that the thesis entitled “**Studies on Reproductive Ecology of *Strychnos nux-vomica* L. (Loganiaceae)**” submitted in partial fulfillment of the requirements for the award of the degree of **MASTERS OF SCIENCE IN FORESTRY (NATURAL RESOURCES MANAGEMENT)** to the Orissa University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Alok Mohanty, Adm. No. 03NRM/16** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged.

(MR MADHAB CHANDRA BEHERA)
CHAIRMAN
ADVISORY COMMITTEE



**ODISHA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
DEPARTMENT OF NATURAL RESOURCE MANAGEMENT
COLLEGE OF FORESTRY
BHUBANESWAR**

CERTIFICATE-II

This is to certify that the thesis entitled “**Studies on Reproductive Ecology of *Strychnos nux-vomica* L. (Loganiaceae)**” submitted by **Alok Mohanty**, Adm. No. **03NRM/16** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for the degree of **Master of Science in Forestry (Natural Resources Management)** has been approved/disapproved by the students’ advisory committee and the external examiner.

Advisory Committee Chairman

Mr. Madab Chandra Behera

Assistant Professor,
Natural Resources Management,
College of Forestry,
O.U.A.T., Bhubaneswar

Members

1. Dr. M. M. HUSSAIN

Professor & Dean
College of Forestry,
O.U.A.T., Bhubaneswar

2. Dr. A. K. SAHOO

Professor,
Natural Resources Management,
College of Forestry,
O.U.A.T., Bhubaneswar

3. Mr. T. L. Mohanty

Assistant Professor
Forest Genetic Resources
College of Forestry,
O.U.A.T., Bhubaneswar

External Examiner

(Name & Designation)

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Date-

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ABBREVIATIONS USED

%	Percentage
hrs	Hours
fig.	figure
µm	Micro meter
mm	Mili meter
amsl	Above mean sea level
GBH	Girth breast height
DOB	Diameter outside bark
m	Meter
X	A random variable under study
n	Number of replications
C. F	Confidence factor
S.S	Sum of squares
S.D	Standard deviation
S.E	Standard error
g	Gram
mg	Miligram
kg	Kilogram
t	Number of treatments
r	Number of replications
Mt	Treatment mean sum of square
Mr	Replication mean sum of square
Me	Error mean sum of square
C.V	Coefficient of variation in per cent
CD	Critical difference
Et.al	And others things
RET	Rare endanger tree
P/O	Pollen to ovule ratio
N	Number

ABSTRACT

The present investigation entitled “**Studies on Reproductive Ecology of *Strychnos nux-vomica* L.**” was carried out at Silvicultural Research Station, Ghatikia; six sites viz. Andharua, Sundarpur, Daspur, Kochila Berena, Deras and Ambakhali of Chandaka-Dampara Wildlife sanctuary and laboratory of the Department of Natural Resources Management, College of Forestry, OUAT, Bhubaneswar during 2016-18. In the first experiment “Morpho-phenological studies” the crown type (columnar to oval), crown spread area ($8.045 \pm 0.703\text{m}$), crown ratio ($76.82 \pm 6.678\%$), leaf constants viz. palisade ratio (5.61 ± 0.45), vein-islet number ($49.64 \pm 4.36 /\mu\text{m}^2$), vein termination number ($165.46 \pm 14.52 \mu\text{m}^2$), anomocytic type of stomata, stomata size ($94.635 \pm 8.025 \mu\text{m} \times 45.094 \pm 3.962 \mu\text{m}$), stomatal index (16.97 ± 1.495) and stomata number ($0.29 \pm 0.03 \text{ per mm}^2$) were established. In the second experiment breeding system study” anther dehiscence and stigma receptivity was minutely observed and found to be partial homogamous and partial proterandous (mixed type). Pollen was tricolpate and pollen ovule ratio was 335:1 per flower. 99.36% pollen was viable and gradually reduces viability after 72 hours of dehiscence (10.08%). The species found to favour cross pollination with only 17.94 to 39.74 % chances self pollination. Bees (*Apis dorsata*) and butter flies were the principal pollinators. Shy and alternate flowering of the species was also revealed. Study of population structure revealed reverse J-shaped curve indicating disturbance and exploitation of mature girth classes. Established natural regeneration ($> 2 \text{ m}$ height) was observed only outside the canopy spread area of mother tree.

CHAPTER I

INTRODUCTION

Herbal remedies for treatment of various ailments are gaining importance every day because their great potency without any side effect. As per World Health Organisation assessment, approximately 80 per cent of global population including nationals of developed nations rely directly or indirectly on medicinal plants for curing different diseases and ailments (Bodeker 1997). The growing demand for natural remedies in national and international markets encouraging pharmaceutical industries to search herbal repositories of nature for new miraculous phytochemicals. We the Indians are very fortunate to have a vast medicinal plant resource. About 3000 plant species were recognised for their medicinal value of which 1700 species have been well documented as medicinal in Ayurveda and Modern Treatise (Prakasha *et al.*, 2010).

Strychnos nux-vomica L. commonly known as strychnine tree is one of the heavily exploited plant of tropical and sub-tropical region. The plant is much prized in India and world for the poisonous compounds strychnine and brucine present in seed and other plant parts. Seed is official in many traditional as well as modern systems of medicine (Kirtikar and Basu 1991). It is a medium sized deciduous (sometimes semi-evergreen) tree native to tropical and subtropical regions of South East Asia and Australia. In India it is commonly found in moist deciduous and semi evergreen forests of West Bengal, Bihar, Maharashtra, Odisha, Central and South India up to an altitude of 500m amsl.

The alkaloids present in the seed are very toxic to human tissue and hence proper detoxification is essential for potential use (Anonymous 1999). The seeds are bitter and used as aphrodisiac, appetizer, anti-periodic, digestive, purgative, and stimulant. They are applied in the treatment of anemia, lumbago, asthma, bronchitis, constipation, diabetes, malarial fever, skin disease, paralysis, muscle weakness, emphysema, colic, insomnia, nervous debility, dyspepsia, diarrhoea, hysteria, mental emotions, epilepsy, chronic constipation, gout, chronic rheumatism, hydrophobia and spermatorrhoea (Victor *et al.*, 2016; Bhati *et al.*, 2012). Apart from seed, other plant part is also used for medicine and other purposes. The pulp of the ripe fruit is used in

treating paralytic affections of palms and foot (Mitesh 2015). The root bark is useful in cholera, snakebite and intermittent fever. The leaves are applied as poultice in the treatment of chronic wounds and ulcers (Nadkarni 1954). Pharmacologically the drug plant has been tested on animals for its antifungal, antibacterial, antioxidant, anti-inflammation, anticancerous, antidiabetic, neuropharmacological properties as well as for the specific actions on gastrointestinal, bone cells and cardiovascular systems (Katiyar *et al.*, 2010; Chitra *et al.*, 2010). Apart from medicinal uses, seeds have also been used as rodenticide, avicide, insecticide, Nematicide and Piscicide (Behera *et al.*, 2017).

Strychnine tree is commercially cultivated in United States, European Union, Fujian, Guangdong, Guangxi, Hainan, North Australia, Taiwan, and throughout tropical Asia (Patel *et al.*, 2012) but the production of seed is far behind from its demand. In India no such cultivation are observed, though some plant populations are exists in private plots in southern states, are of natural origin. Most of the seed demand for domestic use and export were collected from forests by the tribal and peoples of economically backward classes. They often lop the branches or cut the trees for collection of fruits. The availability of seeds of this species is reducing season by season due to increased exploitation, decreased plant population, habitat destruction and erratic bearing because of climate change.

Poor regeneration, ruthless cutting of matured trees for charcoal production and saplings for fire wood, habitat loss and heavy trading of seeds are compelling this poison nut plant to restrict in few areas. Presently this species is sparsely observed in the forests and has been included in the list of rare endangered tree (RET) category in some states like Chhattishgarh (Chaubey and Krishnamurthy 2015; <http://bsienvis.nic.in>), and vulnerable category in some places of Eastern Ghats (Sankar *et al.*, 2008).

Domestication & commercial cultivation of this species is essential for sustainable exploitation of this drug plant. But there are several impediments to its conservation, domestication and evaluation on scientific lines. Successful conservation and domestication requires detailed knowledge of its genetic variations and propagation method for conserving its gene pool, identification of breeding lines, and multiplication of elite genotypes. Perusal of literature has revealed some information regarding its seed treatment and germination but information regarding its breeding

system, intra population variation, natural regeneration etc. is very rudimentary. The literature is mostly focusing on its ethnic uses, phytochemical properties, seed germination etc. No quality planting material and varieties are available for commercial cultivation. Keeping this in view, the present study was carried out to generate information on some of the issues raised above. The brief objectives of the study were:

1. To study the morpho-phenological episodes in *S. nux-vomica*
2. To study the breeding system leading to reproductive success.
3. To study the regeneration status of the species.

CHAPTER II

REVIEW OF LITERATURE

Strychnos, created by Linnaeus in 1753, is a genus of trees and climbing shrubs of the gentian order. The genus is pan-tropical and comprises of 200 species of which about 75 species are found in Africa, 73 species in America and 44 species in Asia (Victor *et al.*, 2016). Five species of genus *Strychnos* are endemic to South India and Andaman Islands (Hill 1917).

Strychnos nux-vomica L. commonly known as Poison nut or Strychnine tree or Quaker button in English; Kapilu or Chipita in Sanskrit; Kajra or Jahar in Hindi; Kuchila in Bengali; Kagodi in Tamil; Mushidi or Mucidi in Telugu and Kuchla or Kochila in Odia (Nadkarni 1954 and Chopra *et al.* 2006). It is a promising drug plant of many traditional systems of medicine including Unani, Ayurveda, Tibetan, Chinese and Homeopathy as well as modern treatise (Kirtikar & Basu 1991 and Ma *et al.*, 2015). It is cultivated commercially in United States, European Union, Fujian, Guangdong, Guangxi, Hainan, North Australia, Taiwan, and throughout tropical Asia (Patel *et al.*, 2012).

2.1. Botanical classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Gentianales
Family: Loganiaceae
Genus: *Strychnos*
Species: *Nux-vomica*

2.2. Conservation status

The species is in high demand for its therapeutic values and there is acute shortage of seeds. Often seeds are collected unscientifically by lopping its branches or cutting down the trees. Young saplings are cut for fire wood by local dwellers. Poor regeneration coupled with habitat loss and heavy trading of seeds compelling this

poison plant to restrict in few areas. Presently this species is sparsely observed in the forests and has been included in the list of rare endangered tree (RET) category in some states like Chhattishgarh (Chaubey and Krishnamurthy 2015) and vulnerable category in some places of Eastern Ghats (Sankar et al., 2008).

2.3. Morphological characteristics

Strychnos nux-vomica is a medium sized deciduous tree. The trunk is tall, thick, straight and cylindrical covered with yellowish-grey to dark grey, smooth and thin barks. Branches are irregular and covered with smooth ash colored bark and shiny dark green young shoots. Leaves are broad, opposite, simple and entire, smooth, short-stalked, blade orbicular to broadly elliptical or ovate, base rounded to cordate, apex shortly acuminate or acute, glabrous and shiny above, minutely hairy especially on veins beneath, 3–5-veined from the base. Inflorescence is terminal cymes, many-flowered and appears with young leaves at the end of branchlets or on axillary shoots. Flowers hermaphrodite, actinomorphic, homogamous, greenish-white coloured, small in size, nectariferous, and emits unpleasant odour. Flower are pentamerous; calyx lobes ovate, outside densely hairy. corolla with a slender tube of 1 cm long, abruptly widening at the throat, outside glabrous, having pubescence at base, lobes narrowly ovate, of 3 mm long, margin thickened and minutely hairy, greenish white to white; stamens inserted at the mouth of the corolla tube altering with corolla lobes. Anthers pale cream in color and five in number. Ovary is bicarpellary with axile placentation. The style is filiform, glabrous and as long as the corolla tube, stigma capitate (head-shaped). Fruit is a globose, indehiscent berry and 1-3 seeded. Seeds are lens-shaped to orbicular or ellipsoid. (Schmelzer & Gurib-Fakim 2008; Somashekhar & Sharma 2002; Chopra & Chopra 2006).

2.3 Distribution and ecology

S. Nux-vomica is native to tropical and subtropical regions of South East Asia and Australia. In India it is commonly observed in moist deciduous and semi evergreen forests of West Bengal, Bihar, Maharashtra, Odisha, Central and South India up to altitude of 500m. Also, observed in plains, shifting cultivation areas, degraded hillocks and up lands in different agroclimatic zones of peninsular and central India (Shiva 1998). In its natural habitat *Strychnos nux-vomica* occurs at the edges of dense forest,

on river banks and along the shores. It is an emergent tree in mature and "undisturbed" forests and thought to be the transition stage of the succession between the pioneers and the "climax" forest. Under favourable climatic condition seedlings can get established in a wide variety of soils ranging from loamy or loamy-sandy soil, lateritic and clayey loam soils but growth is very slow (Pulliah & Rani 2011).

2.4. Seed biology & propagation

S. nux-vomica is a potential medicinal plant for cultivation in Deccan and peninsular India. It can be propagated through seed and vegetative methods but reproduction through seed is more assertive (Bhat *et al.*, 2001).

24.1 Propagation through Seed:

Ripe berries are collected from trees during March-April. The seeds are removed from the fleshy pericarp, cleaned, dried and sorted. 750-800 seeds weigh one kilogram. Seed is orthodox and mesobiotic in nature (Vanangamudi *et al.*, 2006). Seeds are desiccation tolerant, dry seed (10% moisture content) can be hermitically stored at ambient temperature for 30 weeks without losing their viability. Seeds exhibit physiological dormancy and hence slow and erratic germination (10% germination) is recorded from freshly harvested seeds (Sivakumar *et al.*, 2006). Dormancy can be removed (after-ripening) by storing seeds with 10% moisture content at ambient temperature for 30 weeks or by treating with hormones gibberellic acid (GA3) or indole butyric acid (IBA). Treating seeds with gibberellic acid (Soaking seeds in 500 ppm gibberellic acid (GA3) for 24 h), incubation of seeds at 40 °C for 3 days and alternate water soaking (16 h) and drying (8 h) for 14 days, the germination can be improved up to 32%. But 92 percentage of seed germination can be achieved by using after ripened seeds. Growth of seedlings is very slow but it can be improved by using proper growth media (Vanangamudi *et al.*, 2006).

2.4.2 Propagation through vegetative methods:

Macro-propagation through cuttings and root suckers is suitable (Anonymous 2008). Semi-hardwood cuttings are prepared in early summer and kept under moist conditions after treating with rooting hormones. Rooting percentage is quite low, often

less than 25%. Root suckers from mother plant are pricked out and transplanted in polybags for eventual planting out (Somashekhar 2002).

Micro-propagation/ clonal propagation of kupilu can be successfully done through shoot proliferation and hypocotyl culture in suitable media. For shoot proliferation, nodal explants excised from healthy mature trees are cultured on Murashige and Skoog medium with various additions. IAA and NAA were the most suitable growth regulators, both promoting shoot and root formation (Kumar 1990).

For hypocotyl culture seeds were germinated in MS medium supplemented with 1000ppm gibberellic acid (GA₃) under dark condition. Hypocotyls segments of 3mm size are cultured in MS medium with various additions and maintained at 25±1⁰C with 16 hr illumination at fluorescent light. Kinetin is most suitable for shoot formation and NAA is best for root formation (Kumar and Datta 1989).

2.5 Adulterants of Kopilu

Due to high commercial demand and low availability, its seeds are sometimes adulterated by the seeds of *Strychnos nux-blanda* and *Strychnos potatorum* (Turner & Davies 1963). But sometimes it was adulterated unintentionally because of poor identification.

2.6 Pharmacognostic characters of crude drug

Macroscopic and anatomical observations along with chemical analysis are some features used for judging the purity and authenticity of crude drugs of *S. nux-vomica*. Oval shape of seed, presence of unicellular lignified trichomes in testa, collapsed parenchyma, aleurone grains, thick-walled endosperm cells containing plasmodesma and oil globules, are some identifying characters of raw and powdered drug (Evens 2009).

2.7 Phytochemical analysis

Seed is official in traditional systems of medicine as well as modern tretise. Seed of *nux-vomica* is rich in alkaloids, flavonoids, tannins and triterpenoids, glycosides, lignins and steroids (Subbaiah and Savithramma 2014). More than 90

chemical compounds have been isolated from different parts of *nux vomica* but strychnine and brucine are the principal toxic alkaloids. They occur not only in the seeds but also in roots, wood, bark, fruit pulp and hard fruit shells (Akbar *et al.*, 2010). Indian *nux-vomica* seeds contain 2.6 to 3% of total alkaloids approximately, of which 1.25 to 2.5% is strychnine and 1.5 to 1.7% is brucine. The seeds additionally contain chlorogenic acid, loganin (glycoside), and 3% of fixed oil (Mitra and Acharya 2012). A number of minor alkaloids of medicinal importance are also reported and listed in Table-1.

Table-1. Phytochemicals in different parts of *S. nux-vomica*

Plant part	Phytochemicals	References
Leaves	Strychnine, Brucine, α & β - Colubrine, Vomicine, N-methyl pseudostrychnine, Kaempferol-7-glucoside, 7-Hydroxy coumarin, Quercetin-3-rhamnoside, Kaempferol 3-rutinoside, and Rutin.	Joy <i>et al.</i> , 1998 Eldahshan & Abdel-Daim 2015 Thambi & Cherian 2015
Stem Bark	Brucine, Strychnine, Mavacurine. α & β - Colubrine, Loganin, Longicaudatine, Strychnochrysin, Vomicine, Strychnoflavine, Demethoxyguiaflavine, N-methyl-longicaudatine, Pseudobrucine, Pseudostrychnine, 16-hydroxycolubrine, Caffeic acid ester, Fatty acids and Sucrose	Frederich <i>et al.</i> , 2004; Jonville <i>et al.</i> , 2013 Thambi & Cherian 2015
Root	C-mavacurine, Strychnochrysin	Biala <i>et al.</i> , 1998
Flower	Strychnine, Brucine, Vomicine, Icajine and Novacine	De & Datta 1988
Fruit	Strychnine, Colubrine, Brucine, Pseudostrychnine, Pseudobrucine, Strychnine N-oxide, Brucine N-oxide, Icajine, Vomicine, etc. Iridoids: Loganin, Loganic acid, Deoxyloganin, Ketologanin, Secologanin, Glycosides: Salidroside and Cuchiloside	Maji & Banerji 2017
Seeds	Strychnine, Brucine, Brucine-N-oxide, Strychnine-N-oxide, Loganic acid; 4-N-hydroxymethyl strychnidin-17-acetic acid, 10, 11-dimethoxy-4-N-hydroxymethyl strychnidin-17-acetic acid; Stryvomicine, Stryvomine, Isopseudostrychnine, 5-Oxobrucine, 5-Oxopseudostrychnine, 11-hydroxyl-icajine, 10-hydroxyl-icajine; Deoxy-isostrychnine-chloromethochloride, β -colubrinechloromethochloride, α -colubrine-chloromethochloride; Pseudostrychnine, Pseudobrucine, Secoxyloganin, Caffeic acid, <i>p</i> -hydroxybenzoic acid, <i>p</i> -hydroxyphenylacetic acid, Uvaol, Stigmasta-7,22,25-triene-3-ol, Lupeol, 11-oxo- α -amyrin palmitate, Catechol, Maltol, Adenosine; Igasuric acid, Loganin; β -colubrine, Icajine, 16-Hydroxy- α -colubrine, Vomicine, Novacine, Pseudostrychnine, Pseudo brucine, Isostrychnine, Isobrucine, Isobrucine-N-oxide, Isostrychnine-N-oxide, 2-Hydroxy-3-methoxystrychnine; 3-methoxy icajine, 15-hydroxy strychnine, Isobrucine, Isobrucine N-oxide, Isostrychnine N-oxide, 2 hydroxy-3-methoxy strychnine.	Joy <i>et al.</i> , 1998 Patel <i>et al.</i> , 2017 Yang <i>et al.</i> , 2010 Zhao <i>et al.</i> , 2012 Shi <i>et al.</i> , 2014 Zhang <i>et al.</i> , 2014 Cai <i>et al.</i> , 1994

2.8 Medicinal uses

Almost all part of this tree contains alkaloids of medicinal importance but seed is official in many pharmacopeia. The seeds contain poisonous alkaloids Strychine and brucine which are toxic to human as well as ruminants and rodents. Hence proper detoxification is essential for its medicinal use. The Ayurvedic Pharmacopoeia of India recommends detoxified seeds for ailment of paralysis, facial paralysis, sciatica and impotency (Kirtikar & Basu 1991). Seeds are bitter and used as aphrodisiac, appetizer, anti-periodic, digestive, purgative, and stimulant. They are applied in the treatment of anemia, lumbago, asthma, bronchitis, constipation, diabetes, malarial fever, skin disease, muscle weakness, emphysema, colic, insomnia, nervous debility, dyspepsia, diarrhoea, hysteria, mental emotions, epilepsy, chronic constipation, gout, chronic rheumatism, hydrophobia and spermatorrhoea (Nadkarni 1954, Anonymous 1999, Bhati, *et al.*, 2012 and Prasad *et al.*, 2007). Pharmacologically the drug plant has been tested on animals for its antifungal, antibacterial, antioxidant, anti-inflammation, anticancerous, antidiabetic, neuropharmacological properties as well as for the specific actions on gastrointestinal, bone cells and cardiovascular systems (Patel *et al.*, 2017 and Katiyar *et al.*, 2010). Other parts of this plant possess alkaloids of medicinal importance. The root bark is useful in cholera, snake bite and intermittent fever. The leaves are applied as poultice in the treatment of chronic wounds and ulcers (Chitra *et al.*, 2010). Pulp of the ripe fruit is used in treating paralytic affections of palms and feet (Mitesh *et al.*, 2015). Apart from medicinal uses seed extract are used as rodenticide, avicide, Piscicide and insecticide (Gupta 2012 and Ashraf *et al.*, 2010), leaf extract as insecticide and Nematicide (Sivaraman *et al.*, 2014; Arivoli & Tennyson 2012 and Leela *et al.*, 2012).

2.9 Chromosome number

There is a single report of $2n=44$, 24 chromosomes in this species (Mohrbutter 1936) and other chromosomal details like meiotic behaviour, karyotype etc. are unknown. The basic chromosome number reported in genus *Strychnos* is $X = 11$ or $X = 12$ (Gadella 1966)

2.10 Reproductive ecology

Study of different aspects of reproductive ecology like vegetative and reproductive phenological events, floral morphology, pollination studies, breeding system, seed dispersal and natural regeneration etc. are crucial for accessing reproductive successes and adaptability of a species in an environment. The results of such studies have helped in planning & implementing effective conservation and management programmes for many endangered and threatened taxa. Perusal of literature reveals that very limited information is available on the reproductive ecology of tropical and sub-tropical trees. Hence reproductive ecology of some important tree species is reviewed.

2.10.1 Vegetative and reproductive phenology

Phenological events of species are mostly environmentally induced and hence research findings on such parameters plays a significant role in reproductive and ecological research. Reproductive phenological events of species such as bud initiation, flowering and fruiting etc. determines the reproductive success of a species (Soosairaj *et al.*, 2014). Several workers have studied the vegetative phenology of tree species in tropical, subtropical and subtropical forests. For instance, Beniwal (1987) in Arunachal forest; Ansari (1989) in Madhulia forest; Murali (1992) in Madhumali forest and Arjunan *et al.* (1995) in Ambyarh reserve forest, Ralhan *et al.*(1985) in Kumaun Himalayan forests, Murali and Sukumar (1994) in tropical dry deciduous forest of South India etc.

Reproductive events of a species like bud initiation, flowering & fruiting etc. not only gives an understanding about the reproductive success of a species in a set of environmental gradients but also the evolutionary dynamics of the species (Jindal *et al.*1985).

2.10.2 Pollination ecology and breeding systems in tree species

Pollination ecology is one of the synthetic field of biology dealing with research involving ecology, systematics and evolution providing a theoretical frame work for plant breeding, ecological relationship and conservation of species.

Reddi *et al.*, (1983) reported that *Sapindus emarginatus* is monoecious but become dioecious bearing first the staminate, then the pistillate and finally again the staminate flowers with the temporary cessation of blooming between the first two phases. The breeding incorporates both geitonogamy and xenogamy and the allochronic anthesis promotes out crossing. The flowers are visited for pollen and/or nectar by a broad spectrum of diurnal insects. The energy budget of plant permits 70-75% of hand-pollinated flowers to set fruit. Natural fruit set rates well below this could due to insufficient pollinators.

Raju and Rao (2002) studied the reproductive ecology of *Acacia sinulata* and reported that reproductive phenophase completes in summer season. The flowers are small, massed into globose heads and function as units of reproduction. The plant is self-incompatible and exhibits synchronous flowering to facilitate cross-pollination. The flowers are hermaphroditic and monostylous, offering both pollen and nectar as floral rewards. Foragers included bees, wasps, flies, butterflies and thrips. The pod-set rate was 2% only. The low pod-set rate is attributed to a variety of factors. The study suggests that pod and seed yields may be enhanced by introducing manageable bees together with their nesting requirements.

Raju and Ezradanam (2002) studied the pollination ecology and reproductive behaviour in *Jatropha curcas*. They reported that, flowers are monoecious and protandrous. The ratio of male to female flowers is 29: 1. Both flower sexes open synchronously. The sexual system facilitates geitonogamy and xenogamy. The flower visitors include bees, ants, thrips and flies; bees and flies effect geitonogamy and xenogamy, while ants and thrips effect only geitonogamy. The fruiting behaviour indicates that the plant might selectively eliminate the growing offspring, especially geitonogamous fruit to allocate the resources available to the plant, mostly for xenogamous fruit. The ability to self-pollinate through geitonogamy is considered to be adaptive for *J. curcas* for colonization.

Shi-Jin *et al.* (2004) studied the flowering phenology, pollination ecology and breeding system of *Caesalpinia crista* L. in Dinghushan Nature Reserve, Guangdong, China. They reported that the breeding system is self-incompatible, and protogynous xenogamy. Hymenoptera constituted the major group of pollinators. The pollination type is ambophily, the species could be pollinated by wind if the pollinators were unavailable.

Rao and Raju 2002 studied the pollination ecology of *Pterocarpus santalinus* and reported that yellow, bisexual and papilionaceous flowers bloom during dry season. Flower opens at around midnight and offer pollens and nectar for the flower visitors which includes only honey bee. *A. dorsata* was the main pollinator and made visits soon after anthesis under moon light and disappeared at 0730 h. this tree species shows facultative xenogamy but mostly eliminates growing fruits from self pollinated flowers. The fruit set percentage was 6% only.

Kukade and Tidke (2013) studied the pollination and pollen-pistil interaction in *Pongamia pinnata* L. (Fabaceae). It was observed that the flowers are purplish-white, bisexual and nectariferous. The stigma is knob shaped, wet and papillate. The style is of solid (closed) type. The stigma remains receptive during 06.00 to 11.30 am on flower opening day and loses its receptivity shortly before the closure of the flower. The flower remains open only on the day of a thesis. For pollen transfer the plant primarily rely on several bee species such as *Apis dorsata*, *A. cerana indica*, *Amegilla* sp. *Megachile* sp. and *Xylocopa* sp.

Raju *et al.*, 2014 reported that, *Syzygium alternifolium* of semi-evergreen dry deciduous forest in the southern Eastern Ghats of India is a mass bloomer with synchronous flowering during dry season. The floral traits suggest a mixed pollination syndrome involving entomophily and anemophily together called as ambophily. Further, the floral traits suggest generalist pollination system adapted for a guild of pollinating insects. The plant is self-incompatible and obligate out-crosser. The flowers are many-ovuled but only a single ovule forms seed and hence, fruit and seed set rates are the same. Natural fruit set stands at 11%. Bud infestation by a moth, flower predation by the beetle, *Popillia impressipyga* and bud and flower mounds significantly limit fruit set rate. The ability of the plant to repopulate itself is limited by the collection of fruits by locals due to their edible nature, short viability of seeds, high

seedling mortality due to water stress, nutrient deficiency and erratic rainfall or interval of drought within the rainy season. Therefore, *S. alternifolium* is struggling to populate itself under various intrinsic and extrinsic factors.

Raju *et al.*, (2012) studied the reproductive ecology of *Terminalia pallid* and reported that the flowers are bisexual and entomophilous. Cross-pollination is effected mainly by large bees, wasps and butterflies. The natural fruit set is around 6% as against the 62% realized in manual xenogamous pollinations. Fruits fall to the ground when mature and dry, but wind is also instrumental in shedding fruits. The fallen fruits are dispersed by rain water and the seeds germinate and establish seedlings depending on the soil status. Nutrient-poor rocky habitat appears to be an important limiting factor for the recruitment and population expansion.

Shivaprasad *et al.*, (2015) studied the reproductive ecology of *Cinnamomum sulphuratum* and reported that the flowers are self compatible, pollinate both by self and cross pollination. Flower offer both pollen and nectar as a floral reward to the pollinators. Foragers include honeybees, butterflies, wasps, flies and ants. The flowers are self-compatible, pollinate both by self and cross pollination. In allogamy, highest mean percentage of fruit set of 75% was observed.

Smitha and Thondaiman (2016) reported that *Saraca asoca* is cross pollinated. The tree produces bright colour attractive flowers, which changed from yellow/light orange to scarlet/red from the inception of buds to wilting. The bright color of the flowers attracted floral visitors/pollinators thereby facilitated the pollination in this species. Considerable amount of seeds are produced enabling the species to sustain in nature. Polyembryony to an extent of 5% was also recorded in this species.

2.11 Population structure and regeneration pattern study

Understanding of the population structures and regeneration patterns of a tree species assists in designing management practices for maintaining viable population in its habitat. Potential regenerative status of a species within a forest stand is a function of self potency, environmental matrixes as well as stand composition. It primarily depends on three major components: a). ability of seeds to initiate new seedlings/new recruits b) survival of new recruits till attains height up to 2.0 m and c) ability of

seedlings and saplings (established seedlings) to grow (Good and Good, 1972). Measurement of these parameters provides an insight into the regeneration status of species in a forest community.

The regeneration status/ potential of a species in a community are assessed for the population dynamics of seedlings and saplings. The population dynamics are at the pace with which older trees of forest are replaced by younger ones in time and space. Regeneration of any species is confined to a particular range of habitat conditions and major determinant of its geographic distribution (Grubb, 1977). The lack of natural regeneration leads to the degradation of the species from the forests. Several authors have predicted regeneration status of tree species based on the age and diameter structure of their population (Khan *et al.*, 1987 and Bhuyan *et al.*, 2003). Perusal of literature reveals to the fact that, no such work has been conducted for *S. nux-vomica*.

CHAPTER III

MATERIALS AND METHODS

The present investigation entitled “**Studies on Reproductive Ecology of *Strychnos nux-vomica* L.**” was carried out at Silvicultural Research Station, Ghatikia, Khandagiri, Bhubaneswar, Chandaka- Dampara Wildlife sanctuary and laboratory of the Department of Natural Resources Management, College of Forestry, Odisha University of Agriculture and Technology, Bhubaneswar during 2016-18. The research methodology has been detailed under following heads.

- 3.1 Geographical location of the experimental site.
- 3.2 Morphological studies
- 3.3 Phenological studies
- 3.4 Reproductive part studies
- 3.5 Pollination studies
- 3.6 Pollinators, floral visitors and their behaviour
- 3.7 Study of population structure in natural habitat
- 3.8 Natural regeneration and seedlings dynamics studies
- 3.9 Statistical analysis of recorded data

3.1 GEOGRAPHICAL LOCATION OF THE EXPERIMENTAL SITE

The experiment had two main objectives a) to know the morpho-phenology and breeding methodology in *S. nux-vomica* and b) population structure and natural regeneration status in its natural habitat. The morpho-phenological observations and breeding methodology studies were carried out in experimental field of Silvicultural Research Station, Ghatikia, Khandagiri, Bhubaneswar which is situated at 20° 15' North latitude and 85° 52' East longitude at 25.9 m amsl (Fig 1a). The population structure and natural regeneration status was studied in Chandaka-Dampara sanctuary (Fig 1b). The details of annual temperature and rainfall during the experimental period is given in Annexure-I.

Table-2 Details of experimental site under present study

Sl. No	Place	Latitude	Longitude	Altitude (m)
1	Silvicultural research station, Ghatikia	20 ⁰ 27' N	85 ⁰ 78'E	25.9
2	Andharua	20 ⁰ 21' 10" N	85 ⁰ 47' 36" E	56.85
3	Sundarpur	20 ⁰ 20' 25" N	85 ⁰ 46' 37" E	105.6
4	Daspur	20 ⁰ 19' 45" N	85 ⁰ 44' 20" E	85.0
5	Kochila berena	20 ⁰ 18' 36" N	85 ⁰ 42' 14" E	110.0
6	Deras	20 ⁰ 19' 55" N	85 ⁰ 40' 22" E	87.5
7	Ambakhali	20 ⁰ 20' 10" N	85 ⁰ 41' 18" E	98.4

3.2 MORPHOLOGICAL STUDIES

Morphological studies were based on ten randomly selected plants in their natural habitat.

3.2.1 Habit

The habit was studied by observing the general growth features of the plant like height, leaf shedding & flushing, crown spread, flowering period etc. The plant height was measured from base along the axis of the bole to tip of shoot. Diameter of the trunk was measured from base along the axis of the bole to tip of shoot. Diameter of the trunk was taken at breast height (1.37m) and reported as (DOB). The bole height was measured from the base of the tree to the base of the first living branch forming part of the tree crown. Crown spread was measured by taking average of two measurements of the widest spreads at 90 degree to one another. The data was recorded in meters and has been reported as mean.

3.2.2 Type of crown, leaf, stem and root

Various qualitative parameters pertaining to type of crown, branching pattern, root, stem and leaf were studied on the basis of the morphological features of the plant as given by Lawrence (1951).

3.2.3 Leaf

Leaves were studied for different qualitative and quantitative characters. Length was measured including petiole and breadth at widest point and mean was reported in

centimetres. Leaf constants like palisade ratio, stomata type, Index, vein-islet number, vein termination number etc. was determined as per the following procedure.

a) Clearing of leaf

Leaves were boiled for 30 minutes in chloral hydrate solution or till leaves became completely dechlorophyllled. Chloral hydrate solution was made by dissolving 50 grams of chloral hydrate powder in 20 milliliters of water.

b) Palisade ratio

Number of palisade cells below four adjacent epidermal cells in dechlorophyllled leaves was counted. The palisade ratio was calculated on single epidermal cell basis and was based on 10 observations from different leaves. Palisade cells which were less than half inside the observed epidermal cells were not counted while cells which were more than halves were counted.

c) Stomatal studies

The stomatal studies were made from transparent nail polish peel print made of an approximately 1 X 1 m area of leaf between the main vein and lateral vein (Boso *et al.*, 2016) For this a fine layer of transparent nail varnish was applied to abaxial surface and allowed to dry. It was then peeled off with the help of a scalpel, placed on a microscope slide on to which few drops of safranine dye had been placed and covered with a cover slip. Observations were made using Olympus optical microscope Magnus MLX plus (objective 40x). Stomata size and number per unit area of leaf was counted using Magnus Pro3.7 software. The type of stomata was determined on the basis of stomatal classification as given by Fahn (1967). The stomatal index was determined using the following formula based on 50 readings:

$$\text{Stomatal Index} = \frac{S}{(E + S)} \times 100$$

Where,

S = No of stomata/unit area.

E = No. of epidermal cells in the same area.

d) Vein-islet and vein termination number

The vein-islet number is the average number of vein-islets per square millimetre of a leaf surface. Whereas the vein termination number is the number of free vein terminations per mm² in lamina midway between midrib and its margin. Vein-islet and vein termination number was determined by counting the number per one mm² of the leaf lamina between midrib and margin at 4x magnification by using Olympus Optical microscope Magnus MLX plu attached to PC with Magnus Pro3.7 software.

3.2.4 Flower

The floral morphometric studies were made at full bloom stage. Data on inflorescence type, structure and arrangement of various floral parts, length of sepal, petal, stamen, pistil etc. was done on the basis of description of such parameters given by Lawrence (1951), Weberling (1989) and Kaufman *et al.* (1989). Size of flower was recorded with the help of digital calliper (Mitutoyo, model CD-6'' ASX) while as that of floral parts was recorded by using light microscope (Olympus Magnus Plus) attached to PC with Magnus Pro3.7 software.

3.2.5 Fruit and seed

These observations were made at fruit and seed maturity stage on the basis of description of such parameters given by Weberling (1989). Size of fruit and seed was recorded with the help of digital calliper (Mitutoyo, model CD-6'' ASX) and are reported in millimetre.

3.3 Phenological studies

The investigations on major phenological events of the species were carried out for a period of two successive years during 2017-18. A total of twenty individual trees were randomly selected and the observations were recorded with respect to major phenophases viz., vegetative phase, leaf fall, leaf flushing, flowering, flowering duration, fruiting, fruit maturation and dispersal (Newstrom, *et al.*, 1994)

3.4 Reproductive part studies

The floral biology study like anthesis and anther dehiscence, stigma receptivity etc. were conducted during peak flowering season. The observation was taken on

twenty five randomly selected (tagged) mature flower buds of ten inflorescences in five different plants.

3.4.1 Anthesis and anther dehiscence

The anthesis of flowers was recorded from the mature buds of selected inflorescences. Observations like flower opening time and time taken to reach various developmental stages from anthesis to anther dehiscence, mode of pollen dispersal etc. were recorded as per the descriptions given by Dafini (1992).

3.4.2 Pollen production (pollen output/anther/flower) and pollen ovule ratio

The mature anthers were crushed in a cavity block containing ten drops of 1% acetocarmine stain in such a way that all the pollen inside the anther was released in the stain. The stain was steadily stirred so as to make a uniform pollen solution. One drop of this solution was put on a glass slide covered by a large cover slip in such a way that no stain comes out of the edges. This slide was scanned edge to edge in such a way that no pollen was missed or counted more than once. The number of pollen counted per drop was multiplied by ten to calculate the number of pollen grains per anther. These observations were repeated ten times on anthers from different flowers. Finally pollen-ovule ratio was calculated using Cruden's (1977) formula as follows:

$$\text{Pollen Ovule ratio} = \frac{\text{Pollen count per anther} \times \text{No. of anther per flower}}{\text{No. of ovules per flower}}$$

3.4.3 Pollen viability and pollen germination

This was determined by pollen germination test. Few drops of 10 per cent sucrose solution (optimum concentration was ascertained by hit and trial method taking from 1.0 to 15.0 %) was put on a cover slip and fresh pollen dusted on it. This cover slip was inverted on a cavity slide. The slide was kept moist by addition of more 10 per cent sucrose solution placed on the edge of the cover slip. Periodic observations were recorded by counting the number of germinating pollen as well as the total number of pollen per microscopic field and reported in percentage. This experiment was repeated three times taking observation on ten fields each time. The percentage of pollen germination was calculated by using the formula

$$\text{Pollen germination \%} = \frac{\text{Number of pollen grains germinated}}{\text{Total number of pollen grains}} \times 100$$

3.4.4 Stigma receptivity

The stigma receptivity and its duration were determined in the field after anthesis by hydrogen peroxidase activity on stigma. The observation was made by adding a drop of hydrogen peroxide (3%) on stigma of the flower (Dafni *et al.*, 2005). the presence of bubbles or effervescence on stigma surface indicated the stigma receptivity and its duration was observed with the help of 10x hand lense and recorded (Raju *et al.*, 2014 and Sreekala *et al.*, 2011).

Stigma receptivity stage was also determined by pollen germination on stigma surface. Fresh flowering branches with healthy opened flowers were plucked and kept in fresh water. Fresh pollen was physically put on the stigmatic and periodically observed for pollen germination under a light microscope. The stage at which the pollen germinated on the stigmatic surface was determined as the stigma receptive stage. The morphological features of the stigma at receptive stage were noted using a hand lens as well as a dissection microscope.

3.5 Pollination studies

The experiment was conducted under randomized block design with four replications and five treatments. A minimum of 20 flowers were used for each replication. Different pollination mechanisms studied for seed set were as follows.

a). Autogamy

Unopened buds likely to open within next one or two days were enclosed in perforated butter paper bags and left as such without any further intervention.

b) Geitonogamy (Manual self pollination)

Flower buds destined to open in next two days were enclosed in butter paper bags. After opening of the flower, stigma was hand pollinated with pollen from other flowers on the same plant using a fine camel hair brush continuously for 2-3 days.

c) Open pollination (Natural Pollination)

Unopened buds were tagged and left for open pollination under natural condition without any artificial intervention. Fruit set at maturity was recorded.

d) Xenogamy (Manual Cross pollination)

Flower buds likely to open within next couple of days were carefully emasculated without any damage to other parts of flower and enclosed in butter paper bags. After flower opening, the paper bags were removed and pollen from other plants was physically put on the stigmatic region at stigma receptive stage with the help of a camel fine hair brush. After this, flowers were again enclosed in the butter paper bag. This process was repeated for 2-3 days.

e) Apomixis

Flower buds expected to open in next couple of days were carefully emasculated without any injury to other parts of the flower and bagged for fruit setting without fertilization.

Observations recorded

a) Fruit set percentage

It was calculated by counting the number of flowers that set fruit and the number of flowers used for the particular treatment and reported in percentage.

b) Fruit size and weight

Well developed fruits (berries) were measured in two dimensions and the average diameter was recorded in millimetres and reported as mean. Fruit weight was recorded before berries dehisce.

c) Number of seed/fruit

Orange coloured matured berries were collected, fleshy pericarp was removed, washed properly and seeds were dried at room temperature, counted and reported as mean.

d) Weight of seed/fruit

Seed obtained from each berry were separated from all inert material, weighed in gram and reported as mean.

e) Fruit and seed yield per plant

Fruit yield per plant was calculated on the basis of fruit set percentage and seed yield per plant was calculated by multiplying the number of fruit yield per plant and number of seed yield per fruit and expressed in kilogram (taking into account the mean 100 seed weight). This

observation was calculated on the basis of average number of ten thousand inflorescences per plant and fruit set percentage in each treatment.

3.6 Pollinators, Floral visitors and their behaviour

The flower visitors were observed and recorded in peak flowering season during the day time after one hours of anthesis. Observations including the type of pollinators, purpose of visiting, foraging activity and duration of time spent by each pollinator during different hours of the day were made and recorded (Dafni, 1992). The insect pollinators visiting to flower were captured and fixed in 70% alcohol for identification.

3.7 Study of Population structure in natural habitat

Population structure was studied in five different sites of Chandaka-Dampara wild life sanctuary by quadrat method (Mishra, 1968). The number and size of quadrats were ascertained by following the species area curve method given by Mishra (1968). Five quadrats of 10 X 10 m size were laid out at each site keeping a distance of 500 m between any two quadrats. Girths at breast height (1.37 m above ground level) of all nux-vomica trees present were measured. Where main stem is forked below breast height individual stem was measured separately. Data collected were analysed for determining abundance, frequency, basal area, girth class and A/F ratio.

3.8 Natural regeneration and seedlings dynamics studies

Ten numbers of good mother trees having height $\geq 10\text{m}$, GBH $\geq 60\text{ cm}$ and crown diameter $\geq 5.0\text{m}$ were randomly selected in its natural habitat. The selected trees were at a minimum distance of ten meters from each other. The germinated seeds (new recruits) around mother trees were estimated by quadrat sampling. The quadrats were laid around tree trunk in four directions, i.e. north, south, east and west to count and monitor the seedlings that occurred within the tree canopy and outside its canopy spread area. The quadrats of 1 x 1 m were laid from the base of the tree towards the periphery covering a distance of 10 m (Fig.2) (Dalling et al. 1998). The quadrats were placed randomly, one within the canopy spread area (0-5m) and one outside the canopy spread area (6-10m) in all four directions totalling 8 quadrats per tree. All existing

regenerates having height > 2.0 m were treated as established regeneration; below 2.0m were un-established old regenerates and current year seedlings as new recruits. Seedlings within quadrats were tagged and studied for their survival after one year (Chacko 1965). Seed dispersal by birds, squirrels and other animals were omitted from this study.

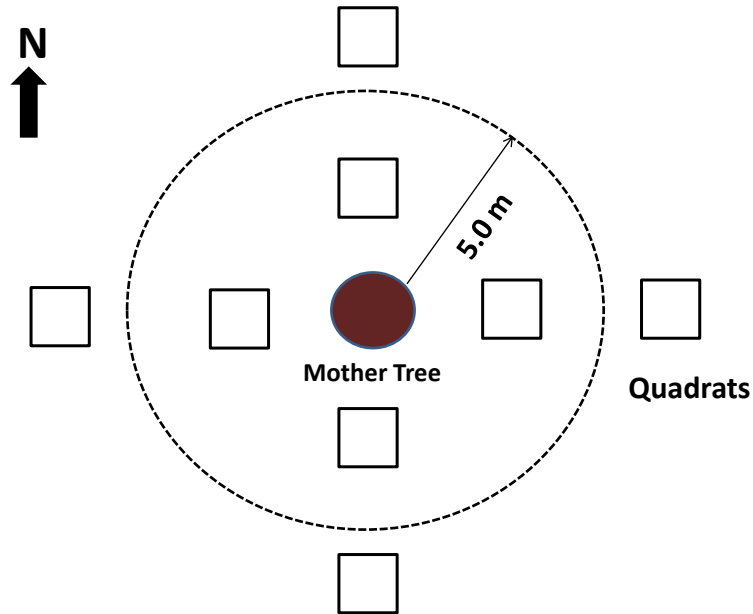


Fig.2 Layout of sample plots (Quadrats) for Regeneration study around mother trees of *S.Nux-vomica*

3.9 Statistical analysis of recorded data

3.9.1 Morphological studies

The data obtained were subjected to statistical analysis as per the methods given by Gomez and Gomez (1984). All parameters studied were statistically analysed for mean and standard error. The data is reported as mean $\pm$ SE (. The standard error is determined by the following method:

$$\bar{x} = \frac{\sum x}{n}$$

$$S.S = \sum x^2 - C.F$$

$$C.F = \frac{(\sum x)^2}{n}$$

$$S.D. = \frac{\sqrt{S.S.}}{n}$$

$$S.E. = \frac{S.D}{\sqrt{n}}$$

Where,

X = A random variable under study

n = Number of replications

C. F.= Confidence factor

S.S. = Sum of squares

S.D.= Standard deviation

S.E. = Standard error

3.9.2 Performance of pollination methods

Pollination methods were evaluated and compared for fruit set percentage, fruit size (mm), fresh fruit weight (g), seed yield per berry (mg), seed yield per plant (kg) etc.. For comparing mean values each parameters was compared by keeping in view the C.D. values at five per cent level of significance. The analysis of variance table was setup as under:

Source of variation	d.f.	Mean sum of squares	Variance ratio
Treatment	t-1	Mt	Mt/Me
Replication	r-1	Mr	Mr/Me
Error	(t-1) (r-1)	Me	

Where,

t = Number of treatments
r = Number of replications
Mt = Treatment mean sum of square
Mr = Replication mean sum of square
Me = Error mean sum of square

The standard error, critical difference, and coefficient of variation were calculated as follows:

$$S.E (d) \pm = \frac{\sqrt{2 \times Me}}{r}$$

$$C.D. \text{ at } 5\% = SE (d) \times t_{0.05}$$

$$C.V. (\%) = \frac{\sqrt{Me}}{\bar{x}} \times 100$$

Where,

S.E.(d)= Standard error of difference

CD at 5%= Critical difference at 5% level of significance

C.V. (%)= Coefficient of variation in per cent

CHAPTER IV

EXPERIMENTAL RESULTS

The results obtained during the present course of investigation entitled “**Studies on Reproductive Ecology of *Strychnos nux-vomica* L.**” were reported under the following heads.

- 4.1 Morphological studies
- 4.2 Phenological studies
- 4.3 Reproductive part studies
- 4.4 Pollinators, floral visitors and their behaviour
- 4.5 Pollination studies
- 4.6 Study of Population structure
- 4.7 Natural regeneration and seedlings dynamics studies

4.1 MORPHOLOGICAL STUDIES

4.1.1 Habit and Habitat

Plants of *Strychnos nux-vomica* L. (Fig.3a) were found naturally growing in plains, shifting cultivation areas, degraded hillocks, up lands, moist deciduous forests and semi-evergreen forests of sub-tropical to tropical regions up to an of altitude 500 amsl. Growth of saplings and trees were found luxuriant in open spaces having overhead sun. But luxuriant growth of new recruits and seedlings were observed in places having partial shade, loamy or loamy-sandy soils. Plants of this species were observed as medium sized deciduous tree having height $9.364 \pm 0.873\text{m}$ (Table-4).

4.1.2 Stem, branch & Crown

Tree axis was orthotropic in nature (Fig.3 c). The trunk was tall, thick, straight and cylindrical in shape, covered with yellowish-grey to dark grey, smooth and thin barks (Table-3). Bole height measured from base of the plant to the first living branch forming tree crown is $2.159 \pm 0.192\text{m}$. The diameter over bark measured at breast height (1.37m) is $65.0 \pm 5.747\text{ cm}$. Branches were mesotonic to irregular and covered with ash coloured bark and shiny dark green young shoots. The crown was broadly columnar to oval. The mean crown spread and crown ratio was $8.045 \pm 0.703\text{m}$ and 76.82 ± 6.678 respectively (Table-4).

4.1.3 Leaves

Leaves were broad, opposite, simple, petiolated, entire, smooth, blade orbicular to broadly elliptical or ovate, base rounded to cordate, apex shortly acuminate or acute, glabrous and shiny above, minutely hairy especially on veins beneath, actinodromous with 3–5-veined from the base (Table-3). The mean length of petiole and leaf was $1.364 \pm 0.122\text{cm}$ & $9.518 \pm 0.844\text{cm}$ respectively. Colour of the leaf in adaxial surface was comparatively greener as compared to abaxial surface (Fig. 4). No special glands or structures were observed. The following leaf constants were observed:

1. Palisade ratio : 5.61 ± 0.45
2. Vein-islet number : $49.64 \pm 4.36 / \mu\text{m}^2$
3. Vein termination number : $165.46 \pm 14.52 \mu\text{m}^2$
4. Stomatal studies :
 - a). Type of stomata : Anomocytic
 - b). Size of stomata : $94.635 \pm 8.025 \times 45.094 \pm 3.962 \mu\text{m}$
 - c). Stomatal index : 16.97 ± 1.495
 - d). Stomata number : $0.29 \pm 0.03 \text{ per mm}^2$

4.1.4 Inflorescence

Inflorescences appear with young leaves at the tip of the previous year shoot branchlets or on axillary shoots. They are terminal cymose type with flowers aggregated closely giving the clustered appearance (Table-3). Flowers are oriented at an angle of 45° to the inflorescence axis and exhibit a skewed distribution (Fig.3 e). Mean length of flowering shoot was 38.464 ± 3.352 and the average number of flowers per inflorescence was 59.182 ± 5.314 (Table-4).

4.1.5 Flower

Flowers were observed to be ebracteate, complete with calyx, corolla, androecium and gynoecium, hermaphrodite, actinomorphic, homogamous, nectariferous, tubular and emits unpleasant odour (Table-3 & Fig 5). Flowers are greenish-white coloured, pentamerous, small measuring $9.836 \pm 0.869 \text{ mm}$ long and $6.873 \pm 0.604 \text{ mm}$ broad with pedicel length of $1.382 \pm 0.123 \text{ mm}$ (Fig3. f).

Flowering bud bursts from 3rd week of February and takes 7-10 days for attaining full size. Flowering (full size) starts 1st week of March (4th March) and

continued till 4th week of April (28th April) with its peak in between 15th March to 18th April. Average numbers per flowers per inflorescence was observed to be 59.182 ± 5.314 (Table-4). Flowers mature and open in succession over a period of 6-7 days with peak on d₃ - d₄(Fig.7).

4.1.6 Calyx

Sepals were observed five in number, gamosepalous, persistent, lobes ovate, tip acute, green in colour, outside densely hairy with odd sepal anterior to mother axis (Table-3 & Fig. 9 a-b). Calyx lobes were measured 3.891 ± 0.341 mm long with the lobes fused up to 0.903 ± 0.082 mm cm from base (Table-4).

4.1.7 Corolla

Petals were five in number, gamopetalous, corolla is in the form of a cylindrical tube with abrupt widening at the throat, outside glabrous, having pubescence at base inside, lobes ovate, greenish white to cream white in colour, stamens inserted at the mouth of the corolla tube exerted altering with corolla lobes (Table-3 Fig. 8b). Mean corolla length was measured 10.982 ± 0.954 mm with tube length 7.424 ± 0.646 mm and Plicae length 3.558 ± 0.310 mm (Table-4).

4.1.8 Androecium

Stamens were five in number, haplostamenous, epipetalous & alternate with petal lobes (Fig.8 e-f). Filaments short and seem to be inserted at mouth of the corolla tube. All stamens were equal in length of 8.855 ± 0.786 mm long. Anthers ditheous, dorsifixed, elliptical in shape, apex exerted (Table-3). Anthers are grouped towards the stigma tip in bud condition. As the flower was about to open, positioned little above the corolla mouth (Fig. 9). Anther length was 0.796 ± 0.07 mm mm and dehisces by longitudinal slits in extrose manner 30-45 minutes after anthesis. Pollens were tricolpate and oblate-spheroidal in shape with a diameter of 41.51 ± 3.623 µm. With 1340 ± 116.187 numbers of pollen grains per anther the average number of pollen per flower was calculated to be 6700 (Table-4).

4.1.9 Gynoecium

Superior gynoecium with bicarpellary syncarpus pistil having bilocular, ovoid, glabrous ovary with 20.00 ± 1.746 anatropous ovule exhibiting axile placentation was observed (Table-3 & 4). Filiform, glabrous cream-white coloured style was 10.915 ± 0.963 mm in length. Capitate stigma tip was papillate, white coloured and $1.38 \pm 0.12 \times 0.39 \pm 0.034$ mm across (Fig 9). Size of ovule was measured $144.476 \pm 12.64\mu\text{m} \times 112.078 \pm 9.921\mu\text{m}$ (Table-4).

4.1.10 Fruit

Single globose, indehiscent berry was produced per flower. Fruit was greenish coloured when immature and orange on maturity (Fig.12 c-h). Exocarp smooth hard but mesocarp was white & fleshy (Table-3). Mean diameter of fruit was 36.49 ± 3.20 mm and weight was 24.117 ± 2.279 gm (Table-4).

4.1.11 Seed

Seed was Lens-shaped to orbicular or ellipsoid. Silky hairs radiating from the centre of the seed gives a shining appearance to the seed. One side of seed was concave and the other was convex with a small depression in the centre of each side (Table-3 & Fig 12 i). Mean seed diameter was $18.065 \pm 1.58 \times 17.81 \pm 1.56$ mm and breadth was 5.89 ± 0.52 mm. The hundred seeds weighed 105.2 ± 9.4 gm (Table-4).

Table 3. Qualitative morphological features of *Strychnos nux-vomica*

Sl. No	Parameter/ Plant part	Character
1	Habit and habitat	Deciduous tree of Tropical and subtropical ecosystem
2	Crown	Broadly columnar to oval
3	Root	Deep tap root system
4	Stem	Thick, straight and cylindrical bole covered with yellowish-grey to dark grey, smooth and thin barks.
5	Branches	Irregular and covered with smooth ash colored bark and shiny dark green young shoots
6	Leaves	Broad, opposite, simple, entire, smooth, short-stalked, blade orbicular to broadly elliptical or ovate, base rounded
7	Inflorescence	Terminal cymose
8	Flower	Many, small in size, greenish-white colored, hermaphrodite, pentamerous actinomorphic, hypogynous, homogamous, nectariferous and emits unpleasant odour.
	i). Calyx	Sepals five, gamosepalous, persistent, lobes ovate, tip acute, green in colour, outside densely hairy
	ii). Corolla	Petals five, gamopetalous, tubular at base and abruptly widening at the throat, outside glabrous, having
	iii). Androecium	Stamens five, haplostamenous, epipetalous, exerted & alternate with petal lobes. Filaments fused with corolla
	iv). Gynoecium	Ovary superior, ovoid, glabrous, carpel two, syncarpous, locule two with axile placentation. Style filiform,
9	Fruit	Globose, indehiscent berry. Exocarp smooth and hard. mesocarp white & fleshy
10	Seed	Lens-shaped to orbicular or ellipsoid. Silky hairs radiates from the centre, One side of seed is concave and the other convex with a small depression in the centre of each side.

Table 4. Quantitative morphological parameters of *Strychnos nux-vomica*

Sl.	Parameter	Dimension/number
1	Plant height (m.)	9.364 ± 0.873
2	Bole height (m)	2.159 ± 0.192
3	Plant Diameter (DOB) (cm)	65.0 ± 5.747
4	Crown Spread (m)	8.045 ± 0.703
5	Crown ratio (%)	76.82 ± 6.678
6	Leaves Petiole length(cm) Length (cm) Width at centre (cm)	1.364 ± 0.122 9.518 ± 0.844
7	Length of flowering shoot (mm)	38.464 ± 3.352
8	Number of flowers per inflorescence	59.182 ± 5.314
9	Flower Pedicel length (mm) Length (mm) Width (mm)	1.382 ± 0.123 9.836 ± 0.869 6.873 ± 0.604
10	Calyx Number of sepals Total length (mm) Fused length (mm)	5 3.891 ± 0.341 0.903 ± 0.082
11	Corolla Number of petals Total length (mm) Tube length (mm) Plicae length(mm)	5 10.982 ± 0.954 7.424 ± 0.646 3.558 ± 0.310
12	Androecium Number of stamens Stamen length(mm) Anther size(µm) Pollen size(µm)	5 8.855 ± 0.786 796.132±69.334 41.51±3.623
13.	Gynoecium Number of carpels Style length (mm) Length of capitate stigma(µm) Width of capitate stigma (µm) Number of ovules Ovule length(µm) Ovule width(µm) Stigma head (µm)	2 10.915±0.963 1383.218±120.324 391.882±34.108 20.00 ± 1.746 144.476±12.64 112.078±9.921 1.38 ± 0.12 X 0.39 ± 0.034
14	Fruit Diameter (mm) Weight(gm)	36.486±3.201 24.117±2.279
15	Seed Diameter (mm) Breadth (mm) 100 seed weight (g)	18.065 ± 1.580 X 17.810 ± 1.560 5.894 ± 0.517 105.2 ± 9.4

4.2 PHENOLOGICAL STUDIES

The phenological calendar of *S. nux-vomia* is given in (Fig. 3 a-h). Leaf fall starts from last week of December and continues till second week of January. Plants remain completely leafless for a period of 7-10 days. Leaf bud bursts second week of February and takes 8-10 days for complete leaf flush. Newly emerged leaves were crimson red in colour and turn light green after 4-5 days of emergence. Mature leaves were dark green in colour and remain in mother plant for eight to nine months. Flower bud bursts with new leaf flush and inflorescences appear during 3rd week of February. Inflorescences attain full size one week after flower bud burst. Terminal cymose inflorescence bears about 59.18 numbers of flowers. Flowering (full size) starts 1st week of March (4th March) and continued till 4th week of April (28th April) with its peak in between 15th March to 18th April. Anthesis takes place during evening hours (15.30 to 15.45 hrs). Flowers mature and open in succession over a period of 6-7 days with peak in between third and fourth day. Corolla withers and detached from the whorl after 12- 18 hours of anthesis. Fruiting commences in April-May and maturation continues till November – December. Orange colour of fruit indicates maturation. Indehiscent berries start falling in the month of January and continued till mid- February (Table 5 & Fig 6).

4.3 REPRODUCTIVE PARTS STUDIES

4.3.1 Anther dehiscence

Anther dehiscence started 30-45 minutes after anthesis which was during evening hours between 15.30 – 15.45 hours. At the time of dehiscence anther lobes reached full length, were at the mouth of corolla lobes or just above that but at almost similar level with the stigma head and anther lobes were creamy white in colour. Dehiscence of anther was through longitudinal slit (**Fig. 7 & 8b**) and continued till next day morning 8.30 hours (16-17 hours after anthesis).

4.3.2 Stigma receptivity:

Stigma receptivity was visibly ascertained by their freshness, viscosity and bubble formation with 3 per cent hydrogen peroxide (Fig. 9d). Stigma became receptive six to eight hours after anthesis and continued for another 2.0 – 2.30 hours indicating partial homogamous development of sexual organs. During the time of anther dehiscence stigma head was slightly above the height of the dehiscing anthers (Fig. 8b). The change

in colour of stigmatic surface from white to light purple colour and senescence of corolla with stamens indicates its loss of receptivity (Table-6 & Fig. 9e).

Table 5. Sequence of events starting from the bud initiation to fruit maturity in *Strychnos nux-vomica*

Number of days since bud initiation	Events
0-2	Floral buds start appearing with calyx lobes closely held together.
3-5	Buds swollen with appearance of corolla. Individual calyx lobes (convex) protrudes out (tip acuminate)
6-8	Corolla tube grows in size and becoming as long as calyx.
9-10	Corolla tube fully elongated, lobes swollen like a bulb at apex, colour deep green at base and fades towards apex. Perianth lobes closed. Anthers held together above the stigma tip.
11-12	Flower opens during afternoon. Corolla colour changed to pale green to greenish white at base to creamy-white at lobes. Petal lobes spread out flat. Anther dehisces longitudinally. Secretion of nectar at corolla
13-14	Anthers are fully devoid of pollen, wither and become black. Capitate head still wet up to 2 nd day of opening. Petal lobes slightly bend down ward from apex. Colour of corolla becomes bright.
15-16	Corolla withers and detached from the whorl and sheds along with exerted stamens. Stigmatic head shrinks in size, becomes dry and colour changes to light pink.
17-18	Filiform style appears swelling, stigmatic head dried and turned black
19-20	Ovary increases in size, filament dries from top
21-26	Set fruit increases to pea size
26-110	Fruit dark green in colour, round in shape, surface smooth and increases in size
111-120	Fruit attains full size and Exocarp slightly rough, and green in colour, mesocarp fleshy and white
121-280	Maturation, no change in size and colour
281-290	Exocarp colour changed to pale green to light orange in colour (physiological maturity)
291-300	Exocarp fully hard, bright orange in colour, mesocarp soft
301-315	Indehiscent berries fully ripen, senescence takes place, & are eaten by birds, squirrels etc.

* Number of days from bud initiation

Table 6. Corresponding sequence of events in development of pollen and stigma receptivity

No. of days since bud initiation	Days from anthesis	Stages of anther/pollen development	Stage of ovary/stigma development
09	1	Flower opens during afternoon hours (15.30 -15.45 hr). Basifixed anthers are slightly above brim of corolla. Anthers dehisces pollen grains longitudinally 30-45 minutes after anthesis.	Capitates stigma surface creamy yellow in colour and slightly above or at par with position of anthers. Filiform style slightly bend, Stigma surface wet but papillae are less prominent.
10	2	Anthers dehiscence continues up to 8.30 am morning.	Style straight, stigmatic surface positioned above the anthers, swollen at both sides, more wet with papillae more prominent. Stigma receptive stage at peak.
11	3	Anthers turn black, and withers towards corolla lobes	Stigma papillae dry and collapsed. Stigmatic head shrinks in size with colour changing to light pink.
12	4	Senescence of corolla along with stamens from flower whorl	Stigmatic head turns black and dried up.

* Stigma receptive for pollen germination

4.3.3 Pollen production, pollen ovule ratio and pollen germination

Shape of pollen grain was observed to be oblate-spheroidal and tricolpate (Fig. 10). According to the result obtained, the pollen grains per anther were 1340 ± 116.187 and pollen grains per flower were approximately 6700. The number of ovules per flower was recorded as 20.0 ± 1.746 and pollen ovule ratio was 335:1 (Table-8).

The percentage of pollen germination at different time intervals after anther dehiscence is presented in Table 7. Maximum pollen germination 99.36 per cent was observed at anther dehiscence stage (Fig. 10d). There was observed a steep fall in pollen germination after 48 hours to 60 hours from 54.91 per cent to 34.27 per cent, respectively (Table-7).

Table 7. Pollen germination in 10 % sucrose solution at different time intervals after anther dehiscence

Hours after anther dehiscence	0 h	12h	24h	36h	48h	60h	72h	84h
Pollen germination (%)	99.36	98.55	80.82	72.82	54.91	34.27	10.18	2.27
S Em ($\pm$)	8.61	8.54	7.01	6.32	4.77	2.98	0.90	0.23

Table 8. Reproductive Characters of *Strychnos nux-vomica*

Sl. No	Character	Description
1.	Flowering period (peak)	15 th March to 18 th April
2.	Flower opening time	Afternoon hours (15.30 -15.45 hr)
3.	Flower type	Bisexual, hypogynous and complete
4.	Flower color	greenish-white colored,
5.	Anther dehiscence pattern	By longitudinal slits and in extrose manner
6.	Anther dehiscence time	30-45 minutes after anthesis
7.	Anther dehiscence time & duration	4.00-4.30 pm 16-20 hours after anthesis
8.	Stigma receptivity duration	36-48 hours after anthesis
9.	Synchrony in maturity of androecia & gynoecia	More or less homogamous
10.	Number of anthers per flower	Five
11.	Number of pollen grains per Anther	1340 $\pm$ 116.187
12.	Number of pollen grains per flower	6700
13.	Pollen viability	98.55 $\pm$ 8.54 (12 hours after anther dehiscence)
14.	Pollen shape	oblate-spheroidal
15.	Pollen aperture number	Tricolpate
16.	Number of ovules per flower	20.00 $\pm$ 1.746
17.	Pollen ovule ratio	335:1
18.	Pollination vectors	Honey bee, butter fly, lady bird beetle, moths, Bumble bee, Ant,
19.	Pollination reward	Pollen grain & Honey
20.	Pollination attractant	Brightness of corolla and odour
21.	Time taken for fruit maturity	290-300 days (9.5 - 10 months)
22.	Seed	Orthodox and mesobic
23.	Mode of reproduction	Seed and cuttings

4.4 POLLINATORS, FLORAL VISITORS AND THEIR BEHAVIOUR

During flowering bees, flies, wasp, butter flies, beetles and birds were observed visiting flowers during day times for collection of nectar and pollen. The time of visit, time spent and reward obtained were given in table-9. Three types of bees *Apis dorsata*, *A. mellifera* and *Trigona spp.* were visited for collection both nectar and pollen. Perusal of data in table -7 reveals that honey bee (*Apis dorsata*) and butter fly (*Episyrphus balteatus* and *Euploea core*) were the principal pollinators (**Fig. 11**).

Table-9 Pollinators, floral visitors and their **activity on *S. nux-vomica* L.**

Insect species	Foraging time (h)	Reward	Number of foraging Visits/day*	Percentage of total visits	Avg. Number of inflorescences visited/ min. **	Length of foraging visit/Inflorescence/Sec.
Bees						
<i>Apis dorsata</i> (Apidae)	7.00-17.00	Pollen & Nectar	165	31	6	7
<i>Apis mellifera</i> (Apidae)	7.00-17.00	Pollen & Nectar	46	9	2	4
<i>Trigona Spp</i> (Apidae)	9.00-11.00	Pollen & Nectar	24	4	2	2
Flies						
<i>Episyrphus balteatus</i> (Syrphidae)	8.00- 16.00	Nectar	68	13	4	8
Wasp						
<i>Vespa Spp.</i> (Vespidae)	10.00-16.00	Nectar	30	6	10	2
Butter flies						
<i>Catopsilia pyranthe</i> (Lepidoptera)	8.00-16.00	Nectar	28	5	4	35
<i>Danaus chrysippus</i> (Lepidoptera)	8.00-16.00	Nectar	34	6	2	20
<i>Euploea core</i> (Lepidoptera)	8.00-16.00	Nectar	65	12	2	30
<i>Junonia iphita</i> (Lepidoptera)	8.00- 16.00	Nectar	42	8	3	45
Beetles						
<i>Coccinella spp</i> (Coccinellidae)	7.00-11.00	Nectar	37	7	2	25
Birds						
<i>Leptocoma zeylonica</i> (Nectariniidae)	8.00 – 12.00	Nectar	12	-	1	3

* 500 anthesed inflorescences were selected to record foraging visits

** Ten observations were made for each insect species

Table- 10 Effect of different pollination methods on fruit set, seed yield and seed viability in *S. nux-vomica*

Parameters Treatments	Fruit set (%)	Fruits per Inflorescence (No.) *	Seed set %	Fruit size (Diameter in. mm) *	Fresh fruit wt. (g) *	Seed / Fruit (No.) *	100 Seed wt. (g)*	Fruit yield / plant (Th. No.) **	Seed yield / plant (Fresh wt.) (Kg.) **	Seed Viability (%)
T₁ (Autogamy)	7.15 (3.67)	3.50 (2.87)	10.75 (4.28)	30.49 (6.49)	26.49 (6.15)	2.15 (2.47)	131.70 (12.48)	35.00 (6.91)	98.96 (10.94)	95.00 (10.75)
T₂ (Geitnogamy)	5.89 (3.43)	3.80 (2.95)	18.75 (5.32)	36.00 (7.00)	32.53 (6.70)	3.75 (2.93)	155.05 (13.45)	38.00 (7.16)	226.05 (16.03)	94.75 (10.73)
T₃ (Open Pollination)	9.775 (4.13)	4.48 (3.11)	21.25 (5.61)	37.34 (7.11)	32.34 (6.69)	4.25 (3.06)	161.13 (13.69)	44.75 (7.69)	157.65 (13.54)	100.00 (11.00)
T₄ (Xenogamy)	10.00 (4.16)	4.68 (3.16)	21.88 (5.68)	37.99 (7.16)	34.11 (6.69)	4.38 (3.09)	164.30 (13.82)	46.75 (7.84)	335.99 (19.33)	100.00 (11.00)
T₅ (Apomixis)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	00 (1.00)	0.00 (1.00)	00 (1.00)	0.00 (1.00)
SEm	0.161	0.112	0.669	0.705	0.320	0.134	1.018	1.115	9.374	0.595
CD_{0.05}	0.497	0.344	2.062	2.172	0.984	0.412	3.137	3.436	28.882	1.834
CV (%)	4.92	6.78	9.22	5.20	2.55	9.22	1.66	6.78	9.99	1.53

Figures in parenthesis are transformed values (Square root x + 1)

* Statistically significant at 0.05 level of significance

Based on an average of ten thousand number of inflorescences per plant

4.5 POLLINATION STUDIES

4.5.1 Fruit set per cent

Maximum fruit set of 10.00 per cent was observed in xenogamy (T₄) as compared to zero and 5.89 per cent in apomixix (T₅) and geitnogamy (T₂) respectively (Table-10). The fruit set per cent in xenogamy (10.00 %) and open pollination (9.775) was statistically at par with each other. The fruit set per cent of 7.15 in autogamy (T₁) was at par with geitnogamy (T₂) with 5.89 per cent (Table-10 & Plate-1)..

4.5.2 Number of fruits per inflorescence

Maximum 4.68 numbers of fruits per inflorescence was observed in xenogamy (T₄) as compared to zero and 3.50 numbers of fruits in apomixix (T₅) and autogamy (T₁) respectively (Table-10 & Plate-3). The number of fruits per inflorescence in xenogamy (4.68) and open pollination (4.48) were statistically at par with each other (Fig. 13a). The number of fruits per inflorescence in geitnogamy (3.80) was at par with autogamy (3.50).

4.5.3 Seed set per cent

Maximum seed set per cent of 21.88 was observed in xenogamy (T₄) as compared to zero and 10.75 per cent in apomixix (T₅) and autogamy (T₁) respectively (Table-10). Seed set of 21.88 per cent in xenogamy (T₄) and 21.25 per cent in open pollination (T₃) were statistically at par with each other. The seed set per cent of 10.75 in autogamy (T₁) was at par with geitnogamy (T₂) with 18.75 per cent (Table 10).

4.5.4 Fruit size

Diameter of spheroidal berries was found maximum in xenogamy (37.99 mm) as compared to zero and 30.49 mm in autogamy respectively. Fruit size of 37.99 mm in xenogamy (T₃) was statistically at par with open pollination (37.34 mm) and geitnogamy (36.00 mm) respectively (Table-10).

4.5.5 Fresh fruit weight

From perusal of table-10, it was found that maximum mean fresh fruit weight was in xenogamy (34.11gm) as compared to zero and 26.49 g in apomixes and autogamy respectively. Mean fresh fruit weight of 37.99 g in open pollination (T₃) was statistically

at par with open pollination (32.34 g) and geitonogamy (332.53 g) respectively (Table-10).

4.5.6 Number of seeds per fruit

Number of seeds per fruit was observed maximum in xenogamy (4.38) as compared to zero and 2.15 in autogamy respectively (Fig.12k). The number of seeds per fruit in xenogamy (4.38) and open pollination (4.25) were statistically at par with each other (Table-10). Minimum seeds per fruit were observed in autogamy (2.15).

4.5.7 Hundred seeds weight

From the perusal of the table-10, it was found that weight of hundred seed was maximum in xenogamy (164.30 g) as compared to 131.70 g in autogamy. The hundred seed weight in xenogamy (164.30 g) was statistically significant from other treatments.

4.5.8 Fruit yield per plant

Maximum fruit yield per plant was calculated in xenogamy (46.75 thousand numbers) as compared to zero and 35.00 thousand numbers apomixix (T₅) and autogamy (T₁) respectively (Table-10). The fruit set per cent in xenogamy (46.75 thousand numbers) and open pollination (35.00 thousand numbers) were statistically at par with each other (Plate-3).

4.5.9 Seed yield per plant

Maximum seed yield per plant was calculated in xenogamy (335.99 kg) as compared to zero and 98.96 kg apomixix (T₅) and autogamy (T₁) respectively. The seed yield per plant in xenogamy (335.99 kg) was statistically significant from other treatments (Table-10 & Plate-2).

4.5.10 Seed viability

Cent percent seed viability was observed in open pollination and xenogamy as compared to minimum of 95.00 per cent in autogamy (Table-10 & Fig. 12j). The seed viability in open pollination (100 %) and xenogamy (100%) were statistically at par with each other.

4.6 Study of Population structure

Mean density, frequency and basal area of seedlings, saplings and trees of *S. nux-vomica* in randomly selected places of Chandaka-Dampara wild life sanctuary and its fringe areas is given in table-11. The density of seedlings, saplings and trees was higher within the sanctuary area as compared to that of fringe villages. Among the fringe village areas seedling, sapling & tree density was maximum in Andharua with 1800 Nha⁻¹, 760 Nha⁻¹ and 70 Nha⁻¹ respectively. The basal area of saplings and trees was highest in Andharua with 0.62m²ha⁻¹ and 2.95m²ha⁻¹ respectively. Within the protected area of sanctuary the density of seedlings (2240 Nha⁻¹) and trees (220 Nha⁻¹) was observed maximum in Ambakhali. But highest number of saplings per hectare (1120 Nha⁻¹) was recorded in Deras. The basal area of saplings was observed highest in Deras (0.903 m²ha⁻¹) and that of trees in Ambakhali (10.31m²ha⁻¹). Density-GBH and basal area-GBH based population structure of within Chandaka-Dampara wild life sanctuary and its fringe areas are given in (Table-12 & Plate 4 & 5).

4.7 Natural regeneration and seedlings dynamics studies

From the perusal of table-09, it was found that a total of 31.75 numbers of seeds per m² were dispersed in 0- 5 m distance from tree trunk within the canopy spread and 8.15 numbers of seeds at a distance of 6 – 10 m from tree bole outside canopy spread area. The number of un-established (< 2.0 m height) old regeneration was 11.60/m² within canopy spread area and 2.08/m² outside the canopy spread area. New recruits (current year seedling growth) were 24.38/m² within the canopy spread area and 3.23/m² outside the canopy spread area. After one year (growth season) survival of new recruits was 9.55/m² within the canopy spread area and 3.3/m² outside of it. The established regeneration was observed to be 0.95/m² outside the canopy spread area of mother trees but no established seedling were found within the the mother canopy. The established regeneration was maximum in south direction (1.60/m²) and nil in north direction. Density-GBH based population curve was found reverse J-shaped (Plate-6).

Table-11 Density, frequency & basal area of *S. nux-vomica* in selected places of Chandaka-Dampara WL sanctuary and its Fringe areas

Site		Seedlings (GBH<10cm)				Saplings (GBH >10-<30cm)				Trees (GBH >30 cm)			
		Density (N ha ⁻¹)	F (%)	BA (m ² /ha)	A/F	Density (N ha ⁻¹)	F (%)	BA (m ² /ha)	A/F	Density (N ha ⁻¹)	F (%)	BA (m ² /ha)	A/F
Fringe areas of sanctuary	Daspur	1080	60.00	0.10	0.075	360.00	20.00	0.39	0.050	20.00	20.00	0.80	0.050
	Sundarpur	920	50.00	0.045	0.092	440.00	20.00	0.41	0.050	40.00	30.00	1.63	0.044
	Andharua	1800	80.00	0.237	0.070	760.00	50.00	0.62	0.020	70.00	60.00	2.95	0.019
Areas within sanctuary	Deras	2040	80.00	0.23	0.080	1120.00	30.00	0.903	0.167	120.00	30.00	4.96	0.133
	Kochila Berena	2160	70.00	0.56	0.110	1080.00	60.00	0.801	0.056	200.00	60.00	5.29	0.056
	Ambakhali	2240	70.00	0.41	0.114	880.00	40.00	0.777	0.213	220.00	50.00	10.31	0.088

F- Frequency of occurrence, BA- Basal Area (1.37m), A/F- Abundance to frequency ratio, GBH- girth at breast height

Table-12 Population structure of *S. nux-vomica* in selected places of Chandaka-Dampara WL sanctuary and its Fringe areas

Site		Seedlings	Saplings		Trees					
		< 10 cm*	≥10 - < 30 cm*		≥ 30 - < 90 cm*		≥ 90 - < 180 cm*		≥ 180 cm*	
		Density (Ind./ha)	Density (Ind./ha)	Basal Area (m ² /ha)	Density (Ind./ha)	Basal Area (m ² /ha)	Density (Ind./ha)	Basal Area (m ² /ha)	Density (Ind./ha)	Basal Area (m ² /ha)
Fringe areas of sanctuary	Daspur	1080	360.00	0.39	20.00	0.796	0.00	0.000	0.00	0.000
	Sundarpur	920	440.00	0.41	30.00	0.973	10.00	0.658	0.00	0.000
	Andharua	1800	760.00	0.62	50.00	1.136	20.00	1.817	0.00	0.000
Areas within sanctuary	Deras	2040	1120.00	0.903	90.00	2.328	30.00	2.630	0.00	0.000
	Kochila Berena	2160	1080.00	0.801	180.00	3.825	30.00	1.463	0.00	0.000
	Ambakhali	2240	880.00	0.777	190.00	6.258	20.00	1.480	10.00	2.575

* Girth class

Table- 13 Regeneration status around mother tree in four directions (per m²) of *S. nux vomica*

Attributes Directions from main trunk	No of Seed Fall (N/m ²)		No. of Old Un-established regeneration (< 2.0m height) (N/m ²)		New Recruits (N/m ²) (Current year seedling growth)		Survival after One year (N/m ²)		Established Regeneration (> 2.0m height) (N/m ²)	
	0-5m*	6-10 m*	0-5m	6-10 m*	0-5m	6-10 m*	0-5m	6-10 m*	0-5m	6-10 m*
East	25.8	7.20	12.00	1.20	37.80	3.40	11.20	2.80	0	0.80
SEm	4.84	1.31	2.39	0.28	6.82	0.85	2.05	0.54		0.20
West	27.6	5.8	11.60	3.40	23.00	5.80	10.20	2.80	0	1.40
SEm	5.34	1.08	2.29	0.75	4.28	1.30	1.86	0.54		0.33
South	44	9.2	15.20	2.60	21.90	2.60	10.40	5.60	0	1.60
SEm	8.33	1.69	2.99	0.57	4.11	0.57	1.87	1.08		0.38
North	29.6	10.4	7.6	1.1	14.80	1.10	6.40	2.00	0	0
SEm	5.57	2.00	1.46	0.28	2.96	0.28	1.22	0.39		0
Total	31.75	8.15	11.6	2.075	24.375	3.225	9.55	3.3	0	0.95
SEm	6.02	1.52	2.28	0.47	4.54	0.75	1.75	0.64	0.00	0.23

* Distance from mother trunk within canopy spread area (0-5m) and outside canopy spread area (6-10)

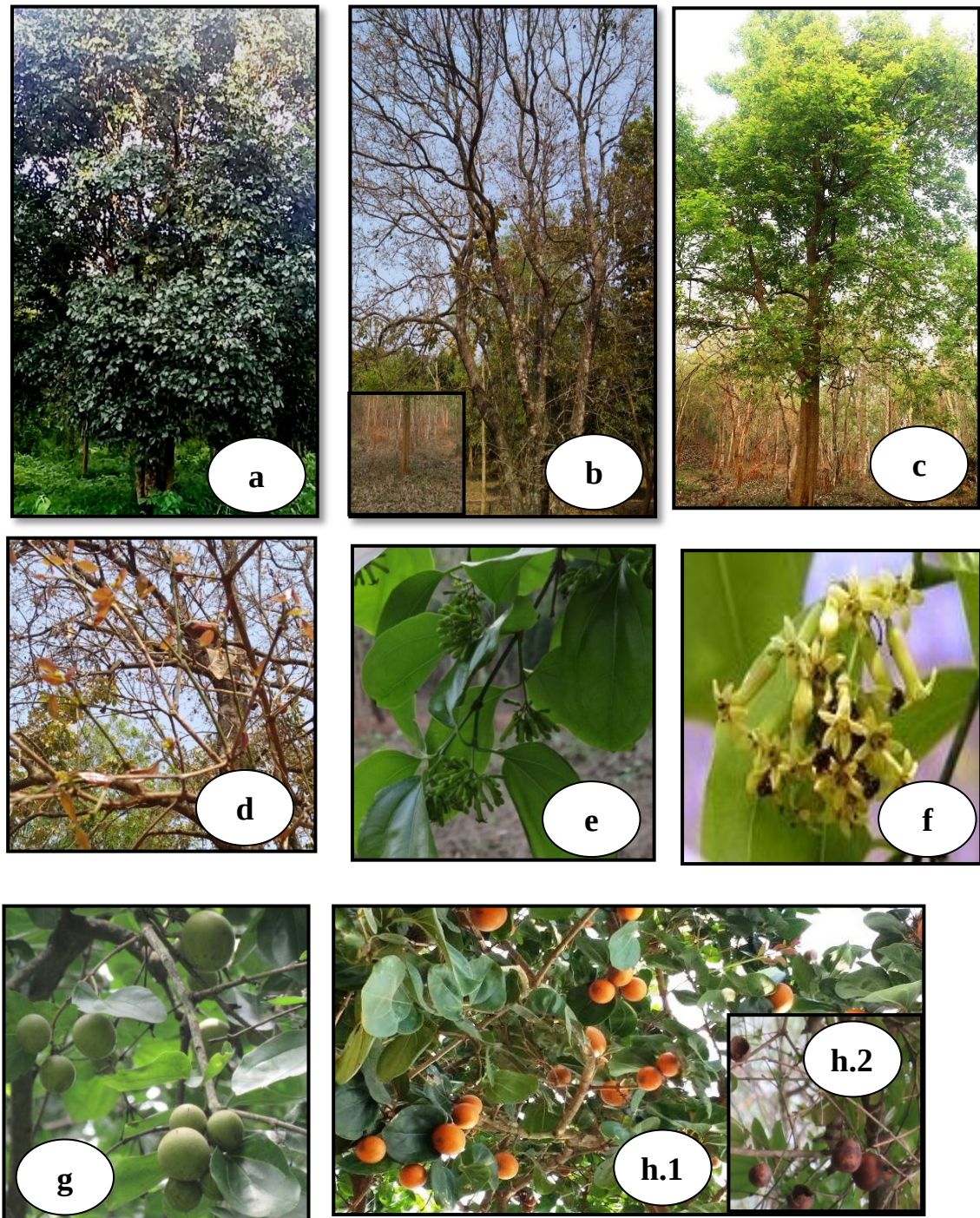


Fig. 3 Vegetative and Reproductive phases of *S. nux-vomica*

- a. Vegetative stage with matured leaves
- b. Leaf fall c. Vegetative stage with new leaf flush
- d. Leaf initiation e. & f. Flowering stage
- g. Immature fruit h1. Mature fruit h2. Seed dispersal

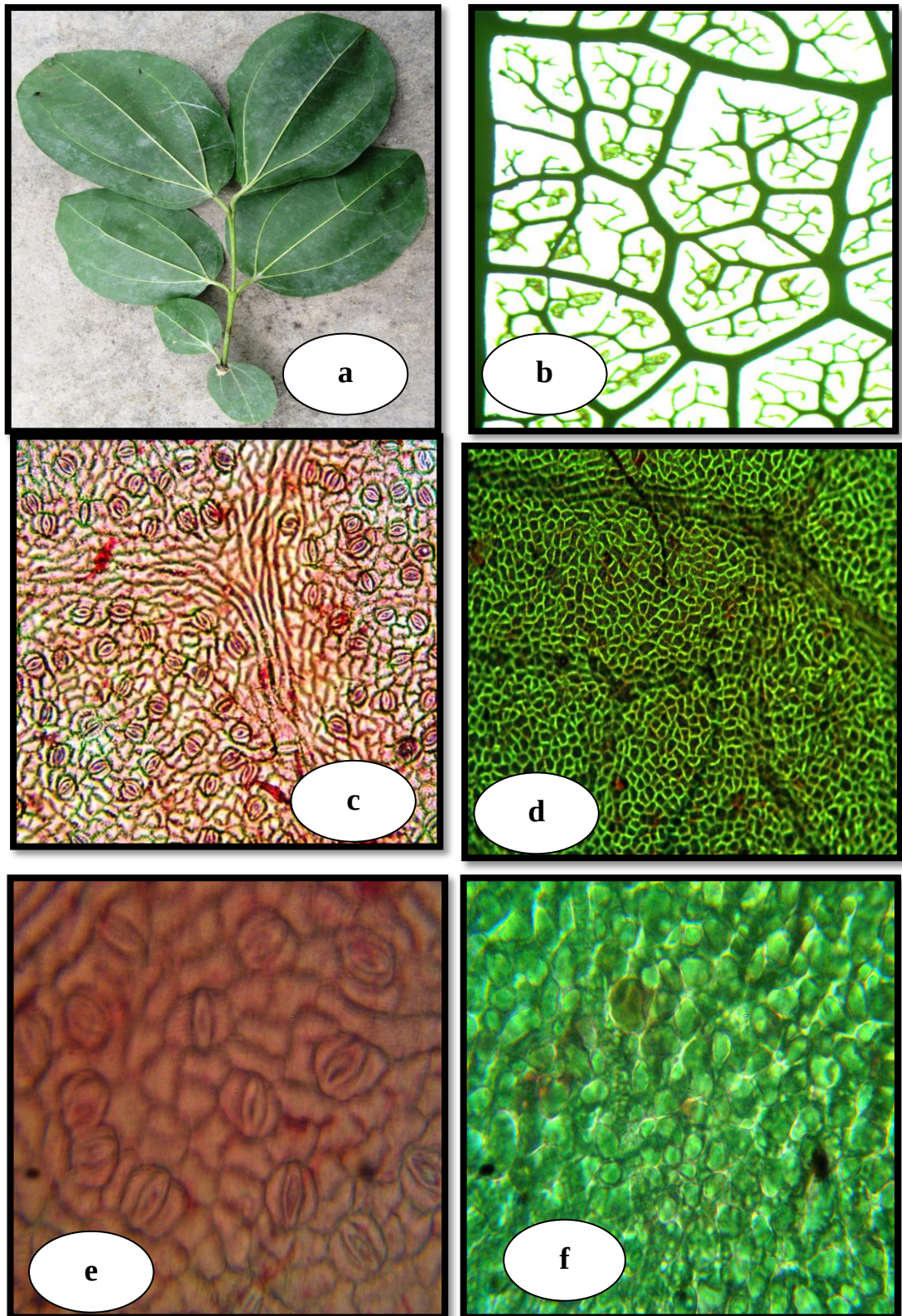


Fig. 4. Leaf morphology of *S. nux-vomica*

- a.** Leaf abaxial surface showing three main veins
- b.** Vein islet & termination
- c.** Stomata along vein region in abaxial surface
- d.** Adaxial surface with no stomata
- e.** Anamocytic stomata
- f.** Palisade cells

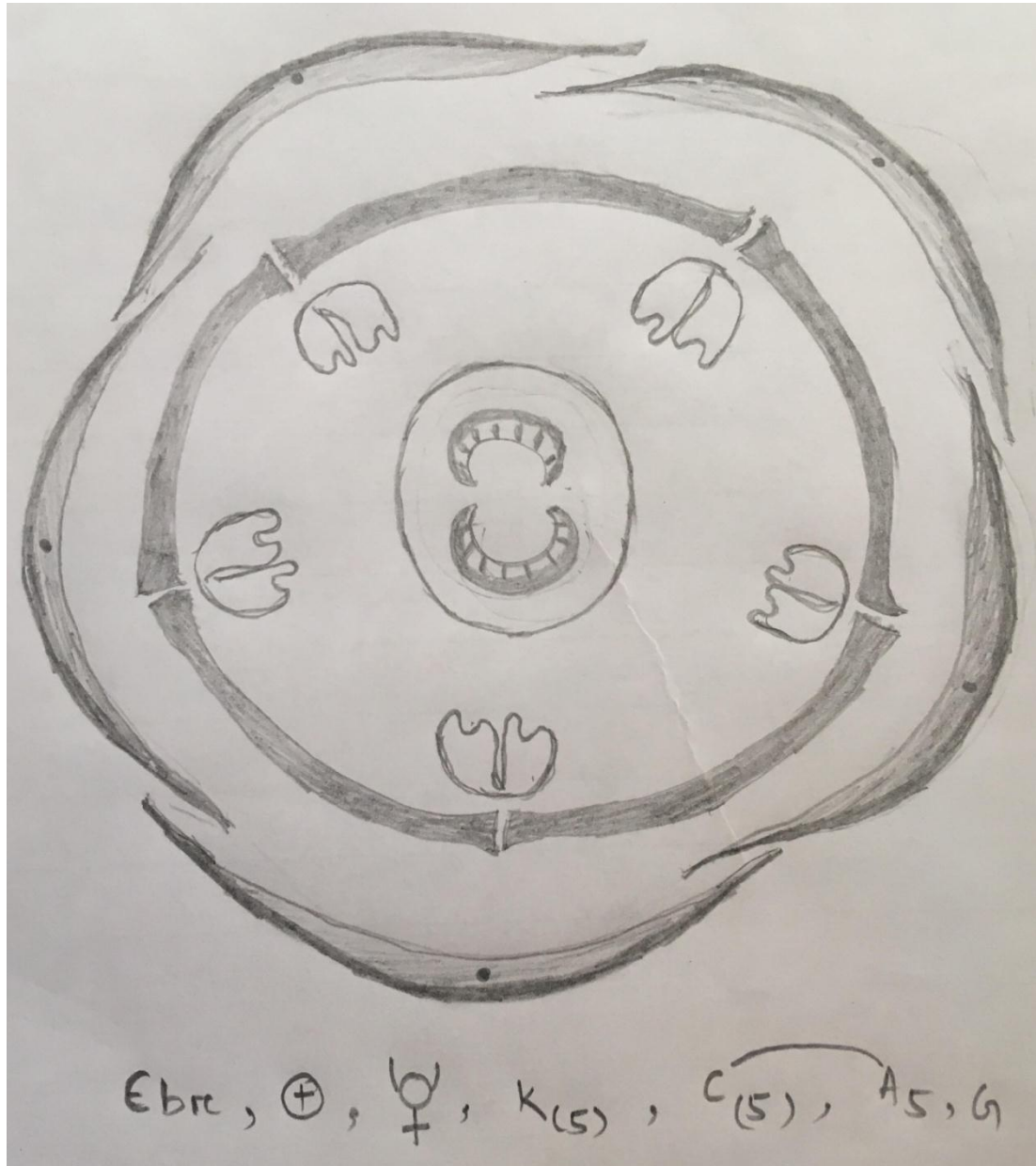
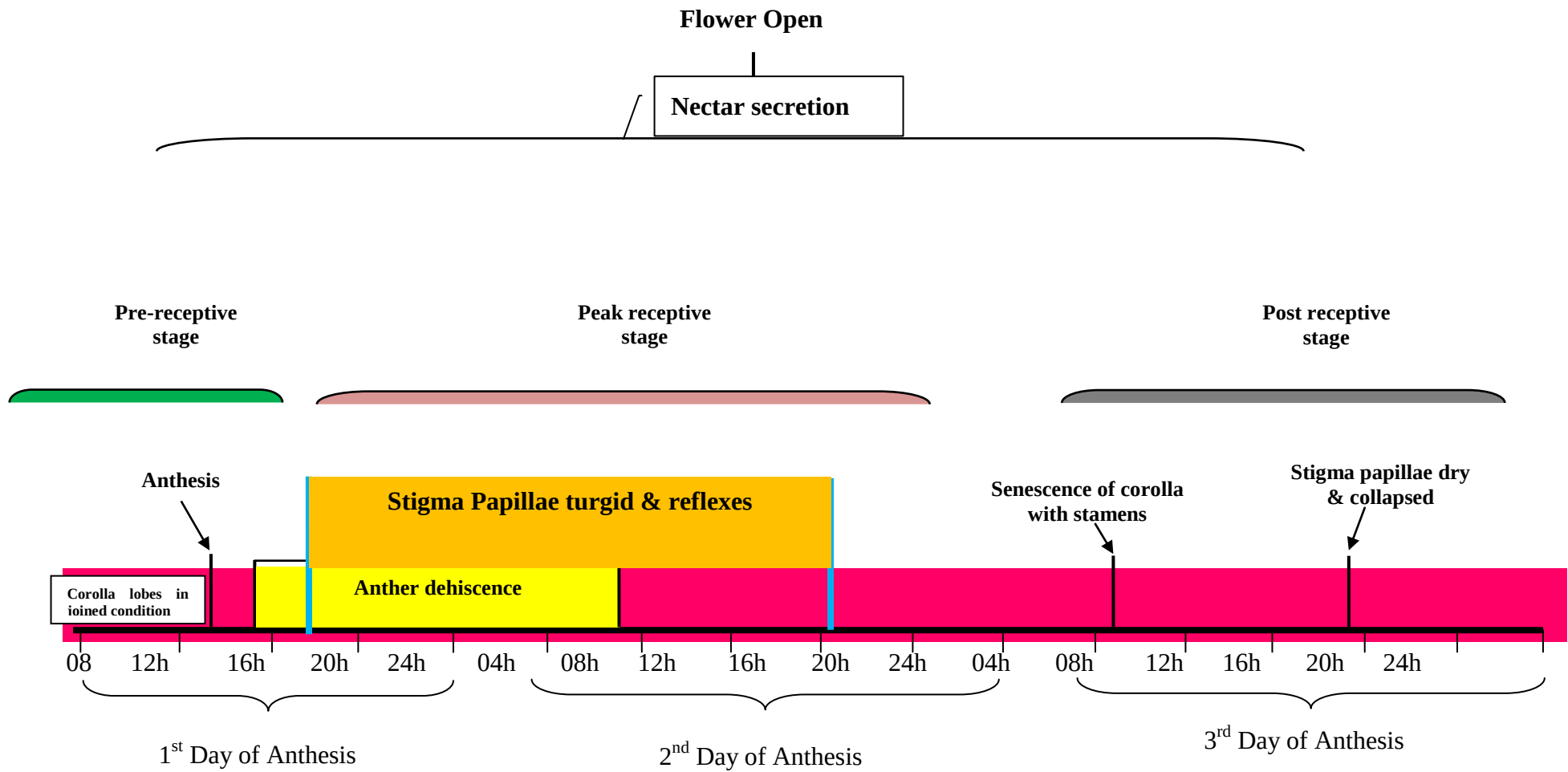


Fig.5 Floral diagram of *S. nux-vomica* L.

Parameters/Months	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Vegetative phase												
Leaf fall												
Leaf flushing												
Flower Bud initiation												
Flowering												
Fruiting												
Fruit maturing												
Fruit fall & seed dispersal												

Fig.6 Phenogram of *S. nux-vomica* L. in Odisha

Fig.7 Phenology of individual *S. nuxvomica* flower during and after flowering opening



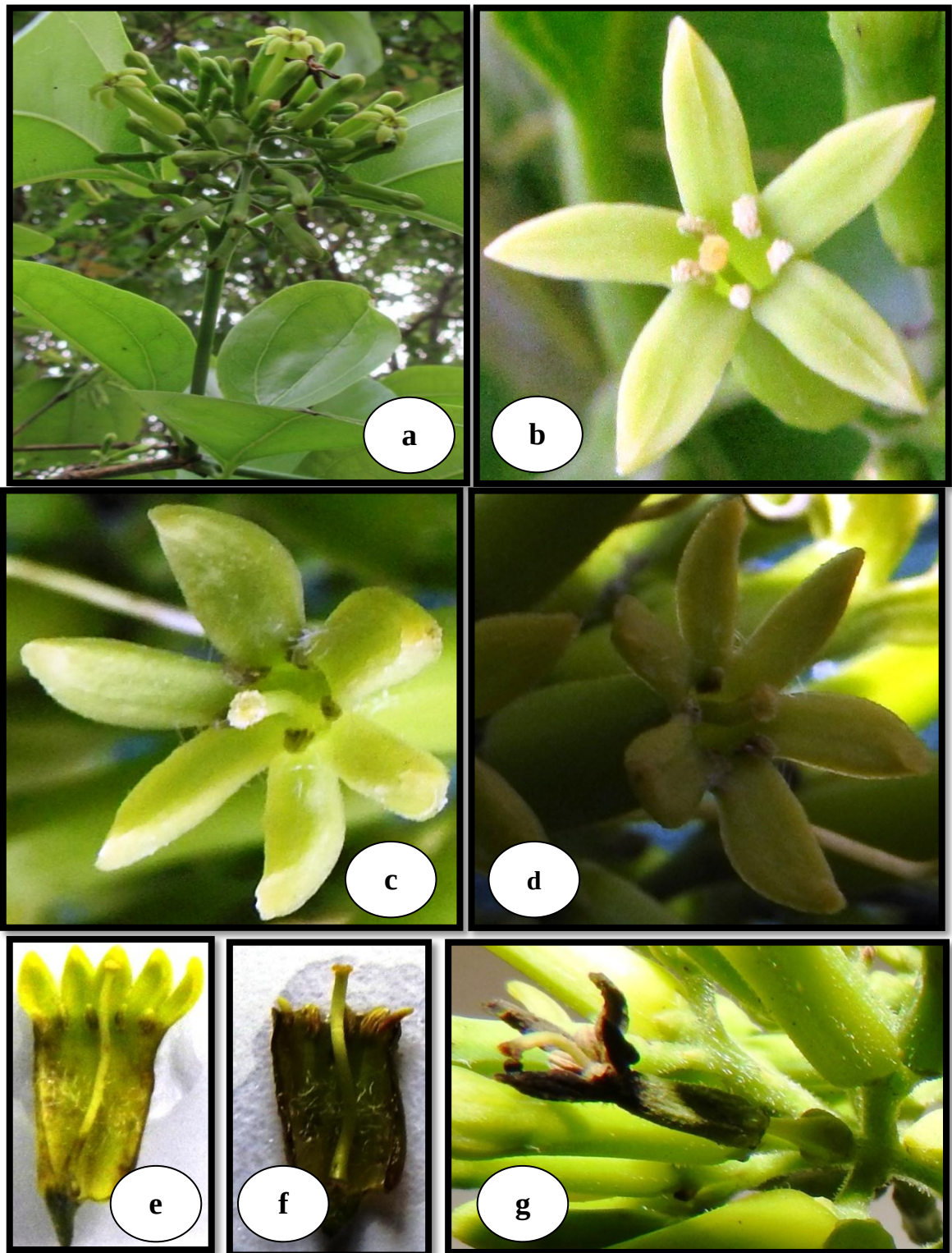


Fig.8 Floral morphological characters (Anthesis, Anther dehiscence & Stigma receptivity)

- a. Inflorescence b. Pollen dehiscence in longitudinal slit
- c. Stigma in active receptive stage after withering of anthers
- d. Stigma in passive receptive stage
- e & f Position of stigma at the time of anthesis and anther dehiscence
- g. Withering of corolla post stigma receptive stage

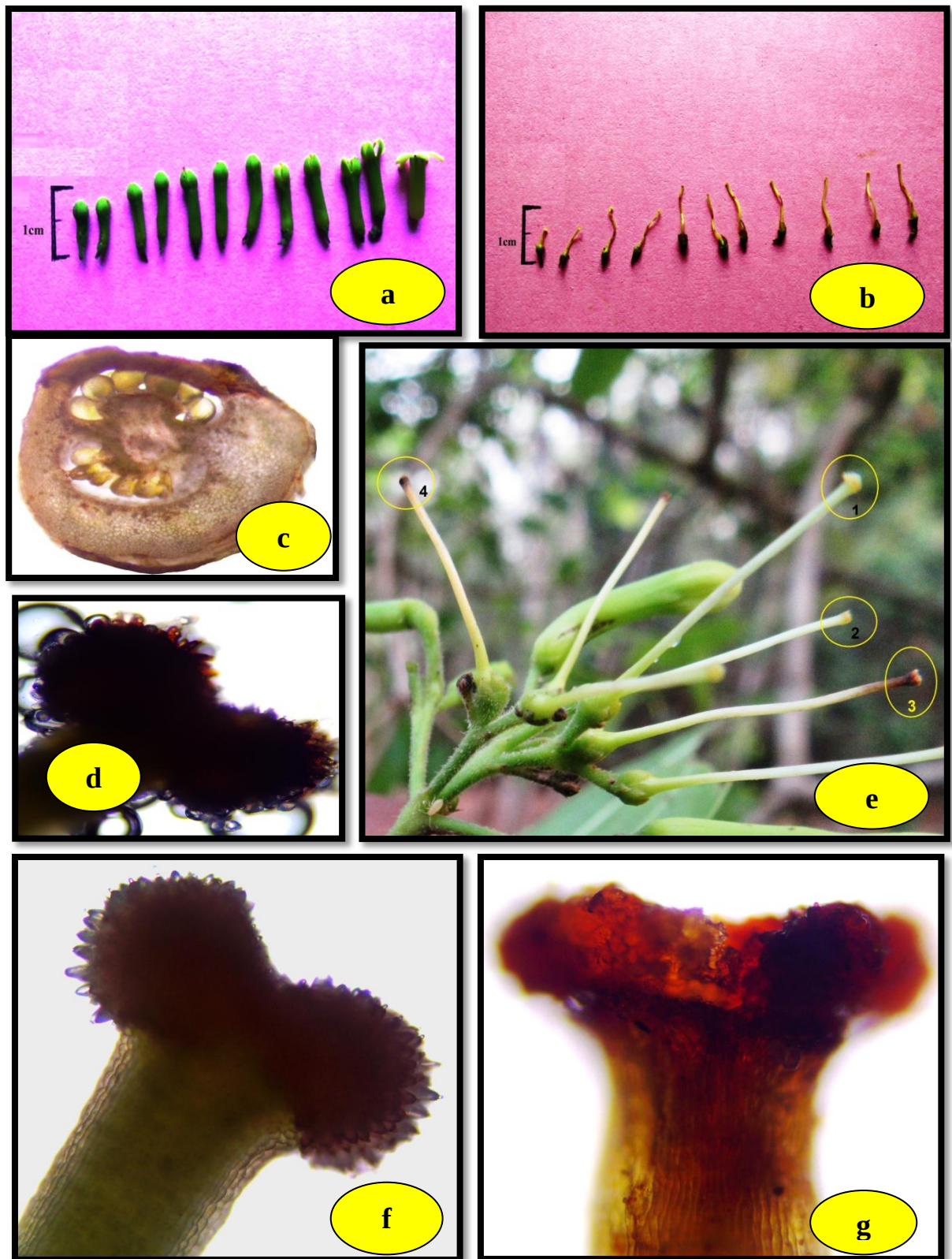


Fig. 9 Stages of flower development & stigma receptivity

- a. Flower development stages
- b. Ovary development stages c. Ovules with axile placentation
- d. Bubble formation with 3.0% hydrogen peroxide
- e. Stigma in 1. active receptive stage, 2- Passive receptive stage, 3 & 4- Post receptive stage
- f. Stigma at Pre-receptive stage with papillae
- g. Stigma at Post-receptive stage without papillae

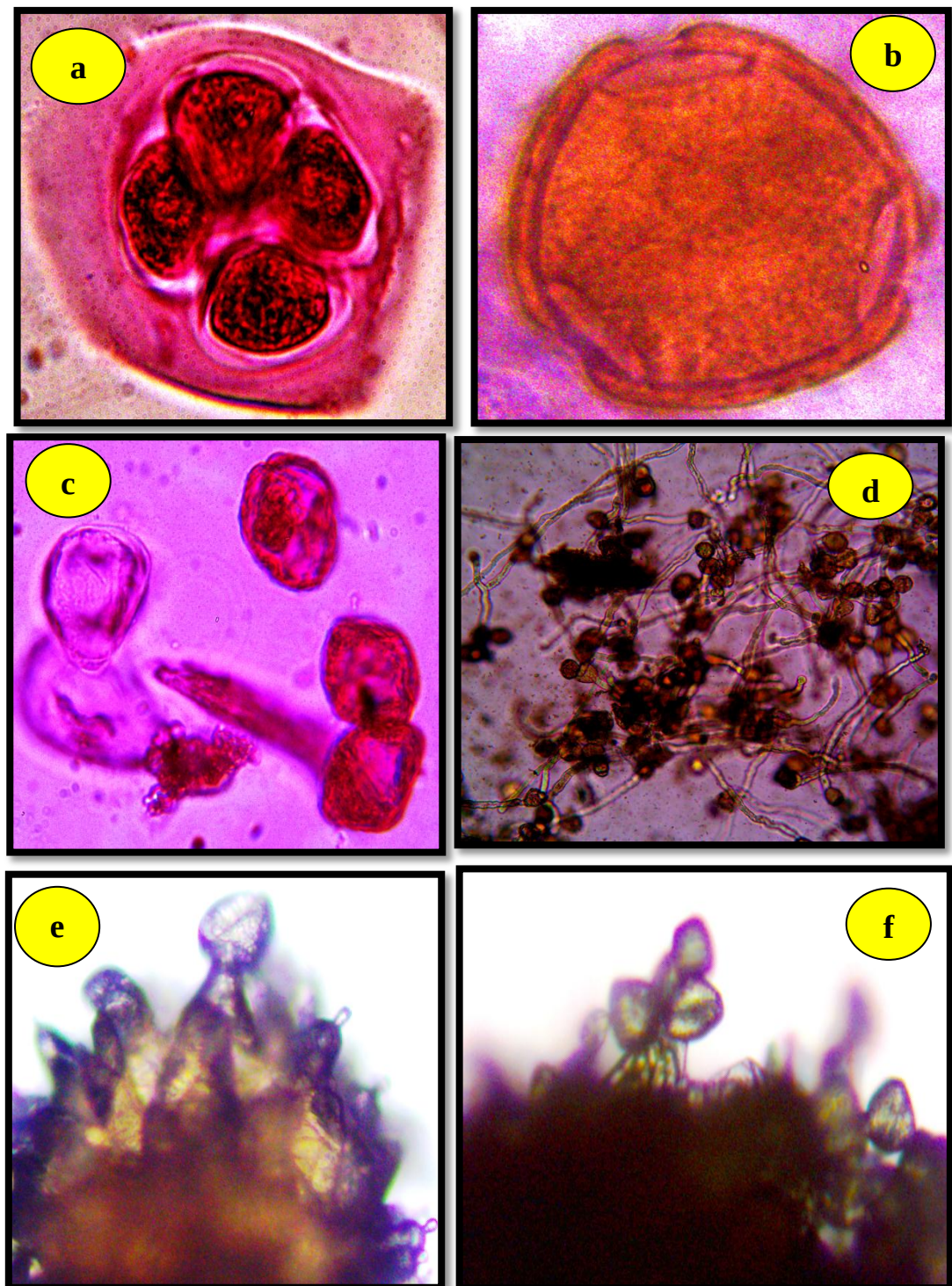


Fig.10 Pollen Viability and germination

- a. Viable pollen tetrad
- b. Tricolpate pollen
- c. Viable and non-viable pollen
- d. Pollen germination in 10 % sucrose
- e. Pollen transfer in stigma
- f. Pollen germination on stigma surface

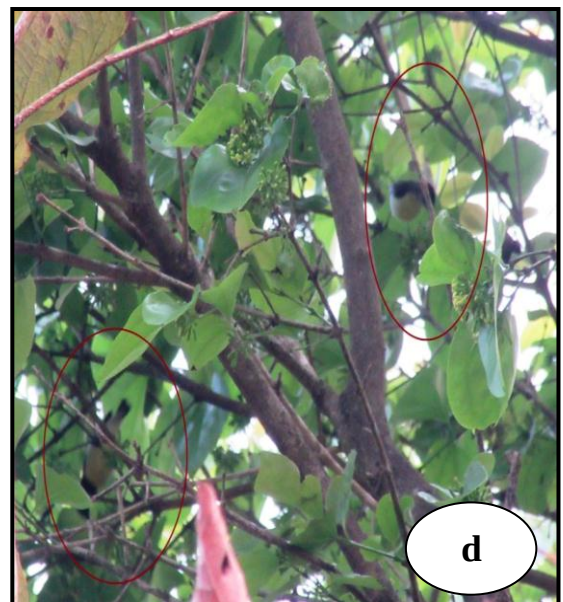
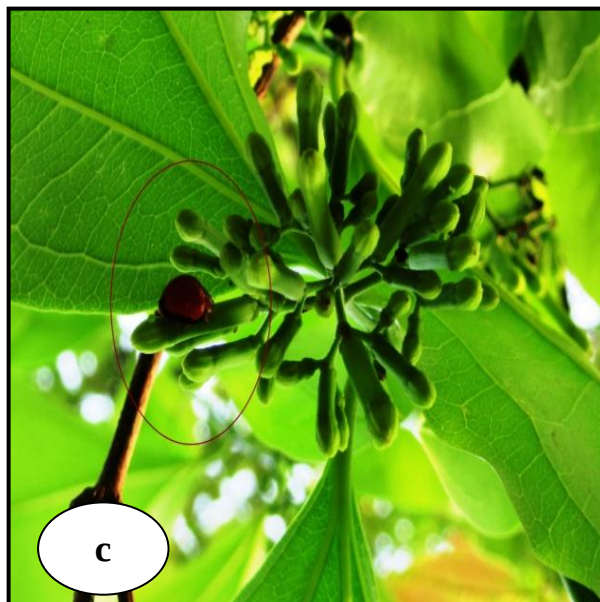
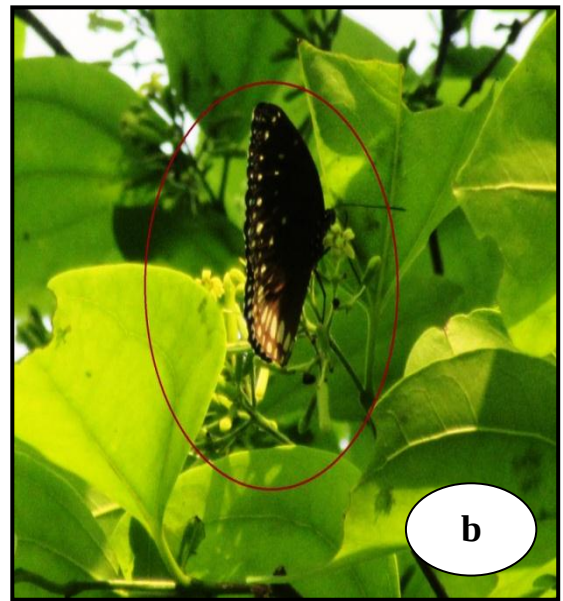
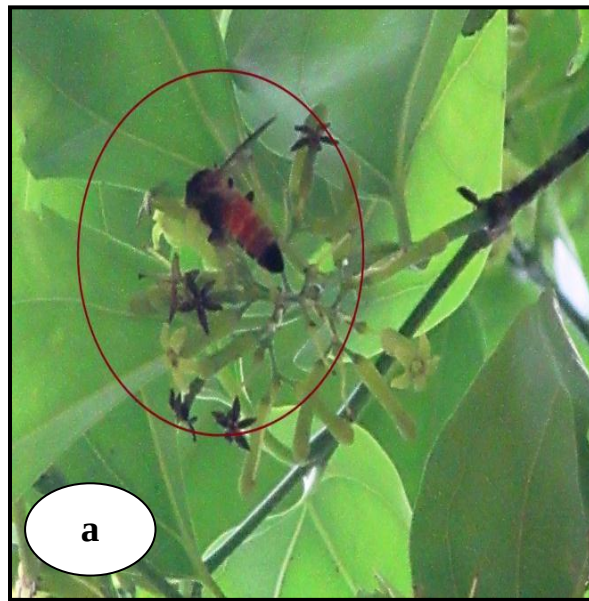


Fig.11 Floral visitors of *S. nux-vomica*

- a. *Apis dorsata* (Apidae)**
- b. *Euploea core* (Nymphalidae)**
- c. *Coccinella* spp. (Coccinellidae)**
- d. *Leptocoma zeylonica* (Nectariniidae)**

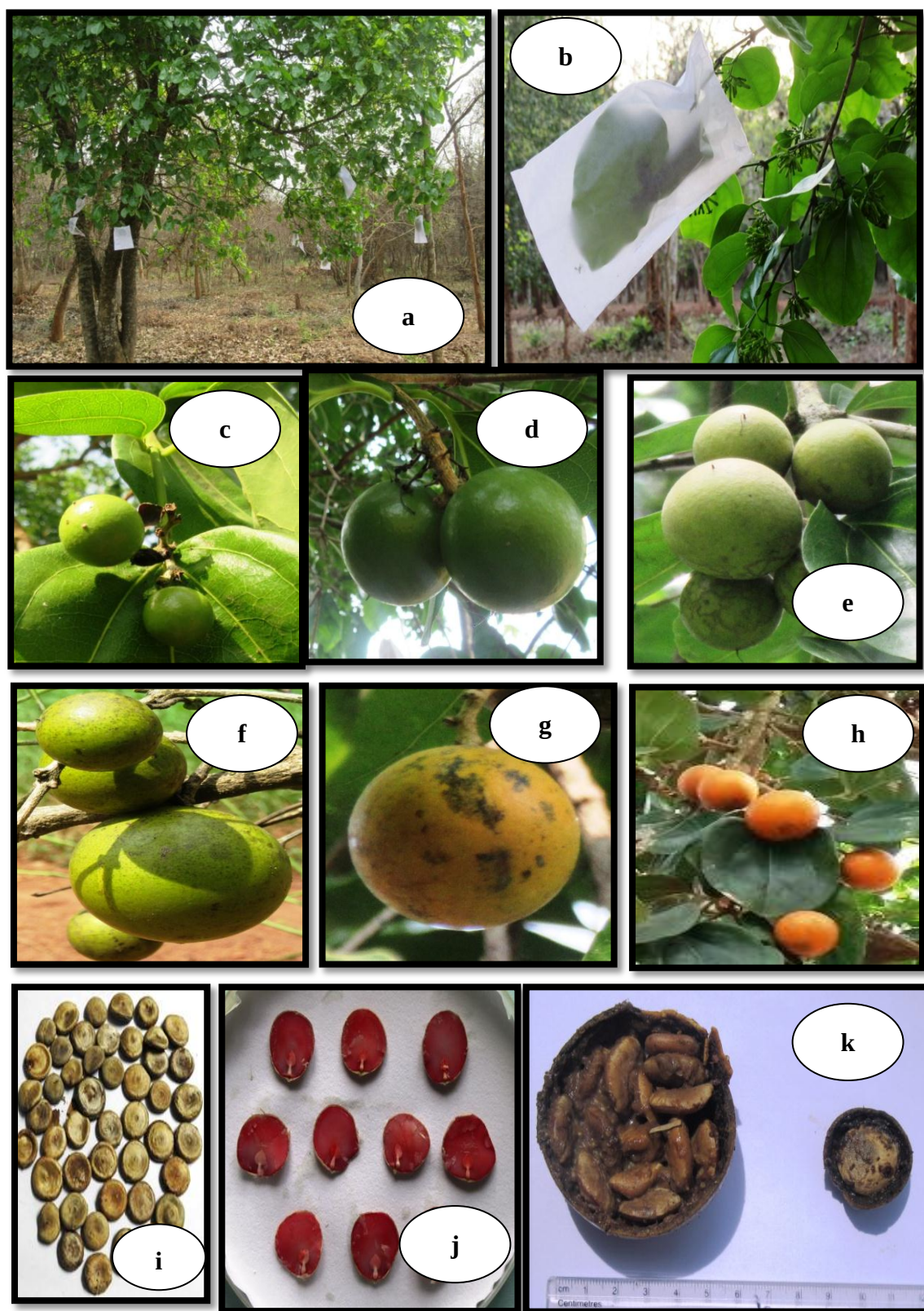


Fig.12. Breeding system & stages of fruit & seed development

a & b Breeding experiment in the field

**c-h Stages of fruit development c- 30 Days after pollination (DAP),
d-60 DAP e – 120 DAP f-210 DAP g-240 DAP h-260 DAP**

i – matured seed j- Viable seeds in 0.5% TTC

k-Variation in number of seeds/fruit

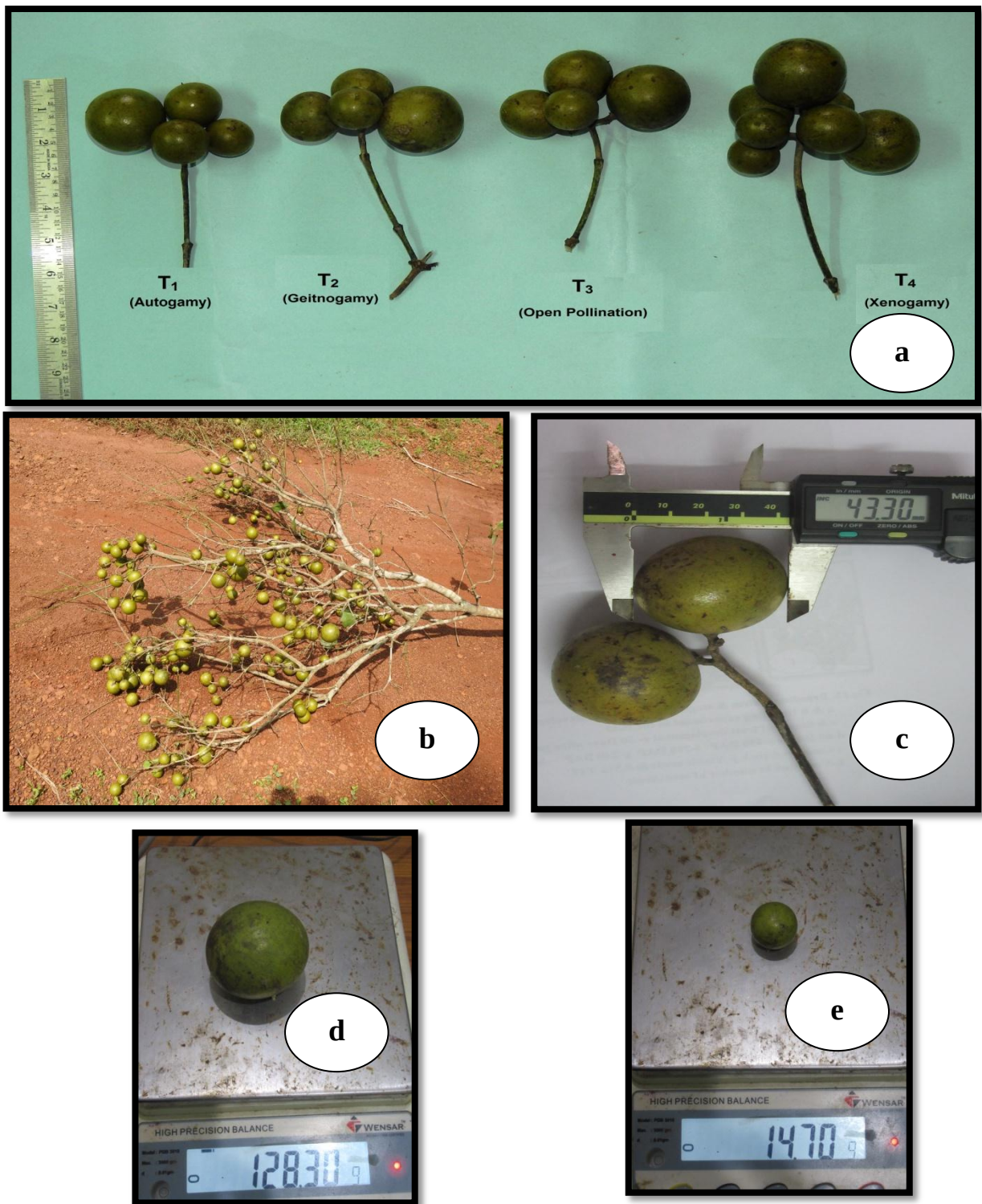


Fig. 13 Impact of pollination methods on fruit set percentage and fruit size

- a. Fruit set per inflorescence
- b Branch with fruit
- c. Fruit size
- d. Large size fruit weight
- e. Small Size fruit weight

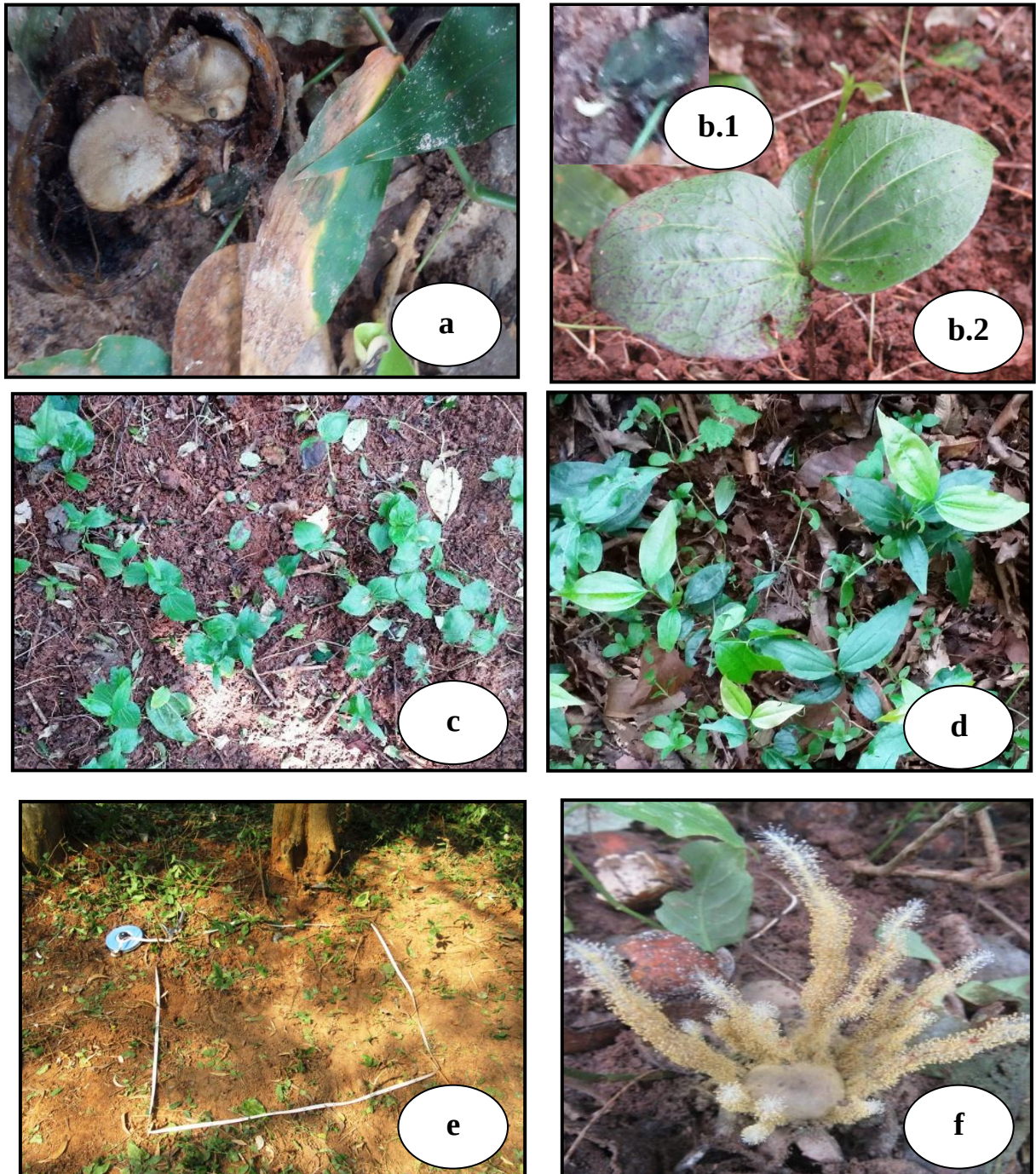


Fig. 14 Natural regeneration

- a. Dispersed seed**
- b1 Seed germination b2 New germination**
- c. New recruits (seedlings of current year growth)**
- d. Old un-established seedlings**
- e. Quadrat method of sampling**
- f. Seed destruction by fungal infection**

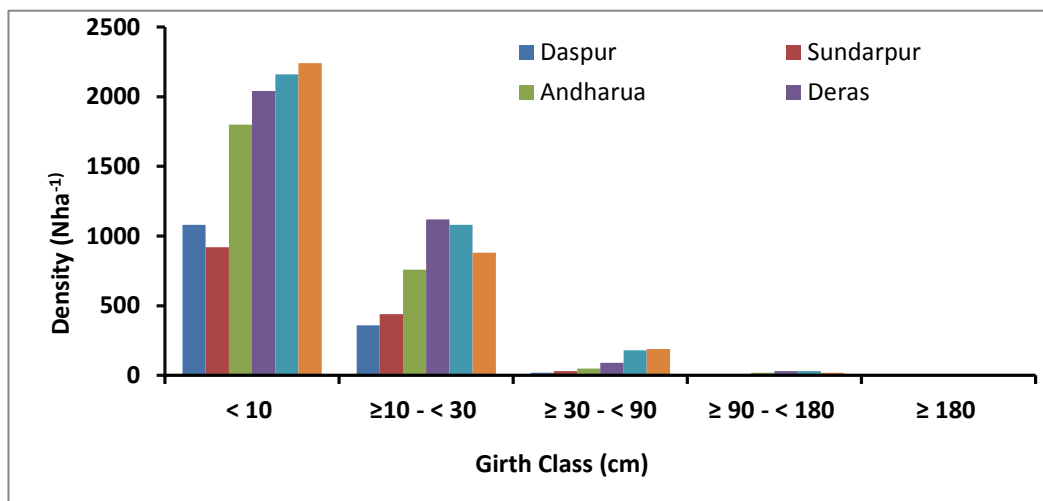


Plate-4 Density Based Population structure of *S. nux-vomica* in within Chandaka-Dampara WL Sanctuary & it's fringe areas

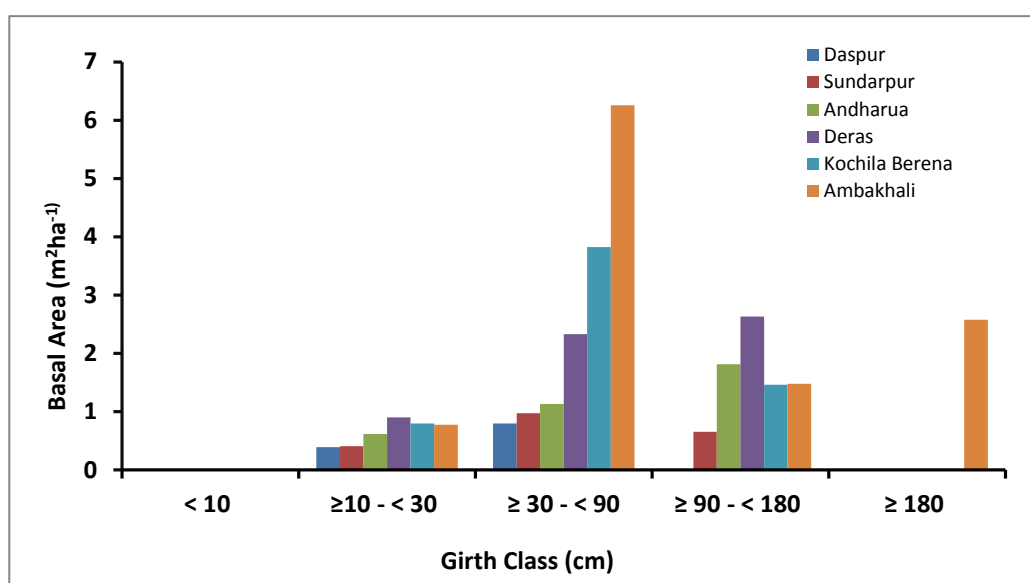


Plate-5. Basal area Based Population structure of *S. nux-vomica* in within Chandaka-Dampara WL Sanctuary & it's fringe areas

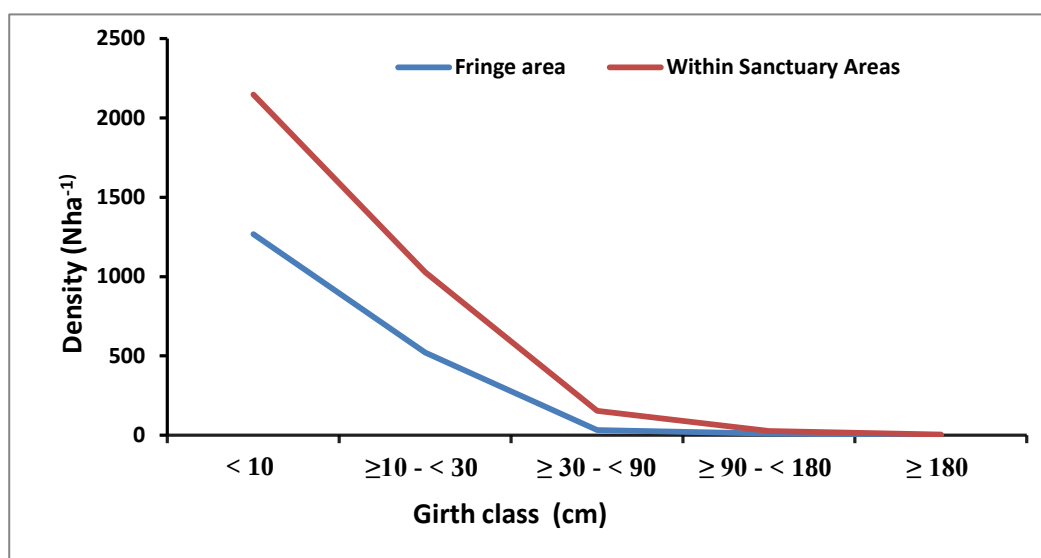


Plate-6 Comparative population structure of *S. nux-vomica* within Chandaka-Dampara WL sanctuary and its fringe areas

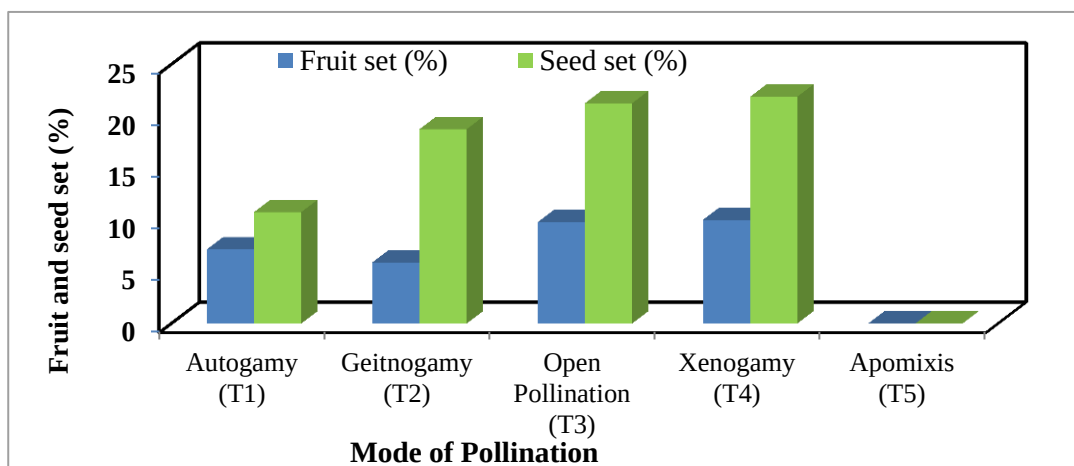


Plate-1. Effect of different pollination methods on fruit and seed set in *S. nux-vomica* L.

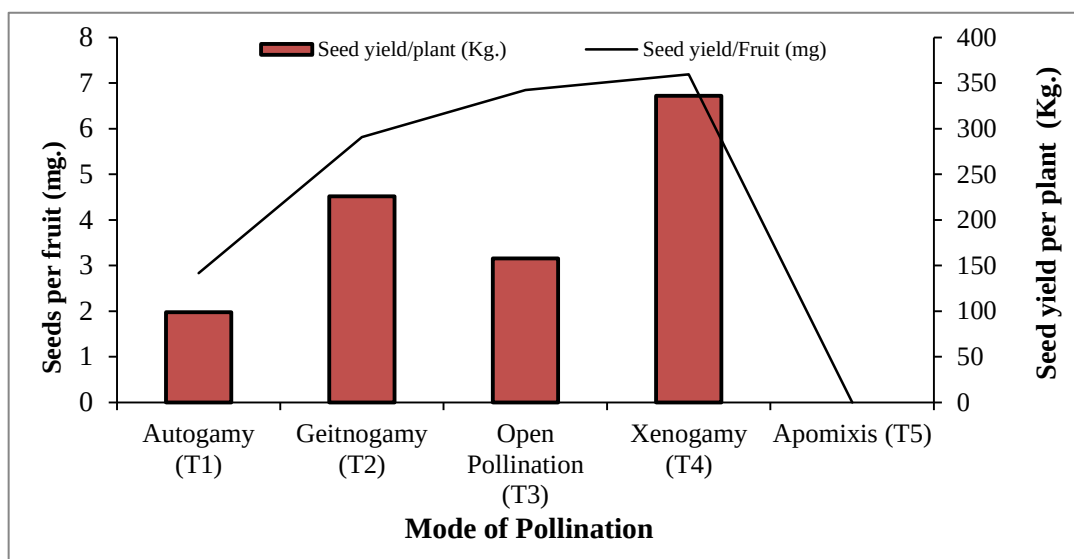


Plate-2 Effect of different pollination methods on seed yield per fruit per plant in *S. nuxvomica* L.

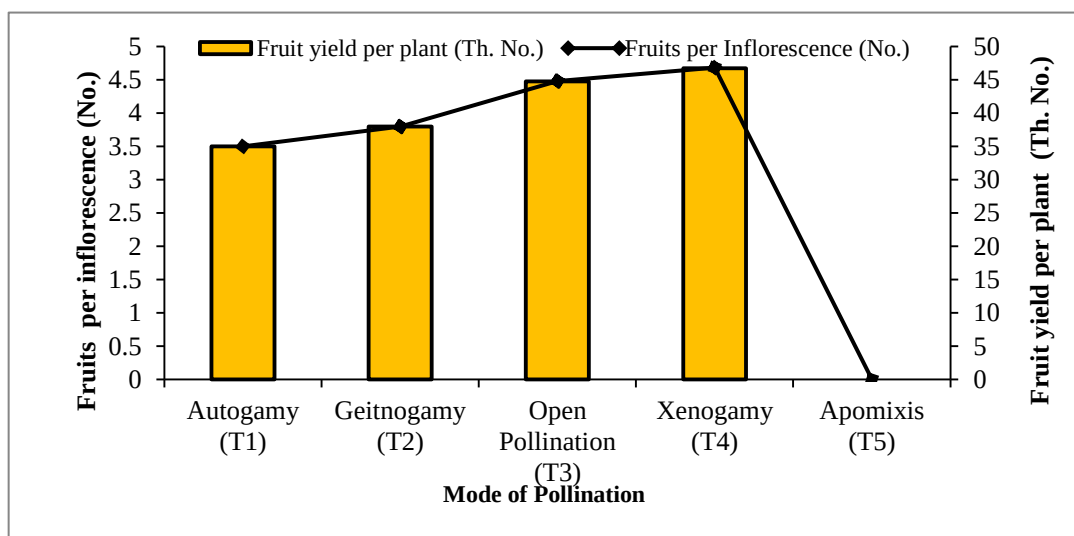


Plate-3 Effect of different pollination methods on number of fruits per inflorescence per plant in *S. nuxvomica* L.

CHAPTER V

DISCUSSION

Strychnos nux-vomica L. is an endemic plant with medicinal properties distributed in the peninsular and central India valued as aphrodisiac, appetizer, anti-periodic, digestive, purgative and stimulant (Victor *et al.*, 2016; Bhati *et al.*, 2012). For these reason seed of this species is extensively collected from its natural habitat. This has lead to scarcity of seeds for natural regeneration and reduction in plant population due to unscientific harvesting, over exploitation, massive felling for charcoal & firewood, and habitat loss. Presently this species is sparsely observed in the forests and has been included in the list of rare endangered tree (RET) category in some states like Chhattishgarh (Chaubey and Krishnamurthy 2015; <http://bsienvis.nic.in>), and vulnerable category in some places of Eastern Ghats (Sankar *et al.*, 2008).

For cultivation and sustainable supply of crude raw materials, suitable agro-techniques of cultivation as well as elite lines are needed for optimal productivity. As in the case with most of forest tree species, genetic improvement has received little attention and the reasons could be many. *S. nux-vomica* is also one of such species where no such work is reported in literature. Perusal of literature reveals that information available are relating to taxonomy, distribution, phytochemical properties and ethno-botanical uses only (Schmelzer & Gurib-Fakim 2008; Nadkarni 1954; Chopra *et al.* 2006) and extensive studies regarding its reproductive potential, ploidy level, breeding system etc. that are essential for its genetic improvement are lacking. Though the species is in high demand its commercial cultivation is absent which could only be possible through adequate scientific knowledge of its reproductive biology, phenology, pollination system, breeding system, seed biology etc. All these studies are pivotal for assessing reproductive fitness of a species to its natural habitat and hitherto to its conservation measures. Phenological and reproductive biology knowledge of a species particularly flowering time, mode of pollination is vital for development of desirable strains and harnessing the quantum of variation present in a species. Keeping this in mind the present work was carried out to generate such information that could be useful in its conservation and commercial cultivation.

5.1 Morphological studies

Morphological studies apart from providing diagnostic inferences necessary for proper identification of a species under investigation, brings new information hitherto unknown. In species like *S. nux-vomica* where traded parts (seeds) are often intentionally adulterated with seeds of *Strychnos nux-blanda* and *Strychnos potatorum* (Turner & Davies 1963), morphological studies are of utmost importance to check adulteration as well as to ensure genuine species for drug purpose. Morphological studies are also imperative for studying inter/intra species variations in a species.

Most of the morphological features of *S. nux-vomica*, studied and documented during present investigations were conformity to earlier reports. Characters like deciduous to semi-evergreen habit, branches covering with smooth ash coloured bark and shiny dark green young shoots possessing simple, opposite three veined leaf, terminal cymose heads, flower and fruit characters observed during this present studies matched perfectly with earlier reports (Saxena and Braham 1994; Haines 1961). The branching pattern is not truly irregular as reported by Thambi & Cherian 2015 but it was observed to be mesotonic to irregular (Barthelemy and Caraglio 2007).

There is no literature record for some essential silvicultural parameters like nature of stem, type of crown, crown spread area, bole height etc. which helps in proper management in a stand. The orthotropic nature of tree axis, bole height ($2.159 \pm 0.192\text{m}$), broadly columnar to oval crown having spreading area of $8.045 \pm 0.703\text{m}$, crown ratio of $76.82 \pm 6.678\%$ were reported for the first time.

Also there is no literature record for leaf constants like palisade ratio, vein islet number, vein termination number, stomatal type, index, number, size etc. in this species, which are important pharmacognostic parameters for any drug plant. These were studied for the first time during present study.

1. Palisade ratio : 5.61 ± 0.45
2. Vein-islet number : $49.64 \pm 4.36 / \mu\text{m}^2$
3. Vein termination number : $165.46 \pm 14.52 \mu\text{m}^2$
4. Stomatal studies :

- a). Type of stomata : Anomocytic
- b). Size of stomata : $94.635 \pm 8.025 \times 45.094 \pm 3.962$
- c). Stomatal index : 16.97 ± 1.495
- d). Stomata number : 0.29 ± 0.03 per mm^2

The type of stomata observed was *anomocytic* type, wherein each stomata is surrounded by similar subsidiary and epidermal cells (Fig.4). This type of stomata is common to taxa belonging to family Ranunculaceae, Malvaceae, Scrophulariaceae, Cucurbitaceae, Geraniaceae, etc. (Fahn, 1967).

All living forms on this planet have evolved and evolution has taken place from simple to complex forms. We also know that evolution has not reached a dead end and all life forms continue to be in a state of evolution. While analysing the features associated with evolution one often encounters primitive and advanced characters. Primitive characters are those which are present in the progenitors and advanced characters represent the manifestations of evolution (Kaufman *et al.*, 1989).

According to Cronquist (1968), there are many characters in living forms which can be considered advanced over primitive characters. Based on the classification of primitive and advanced characters given by Cronquist (1968), *S. nux-vomica* seems to possess the following primitive and advance features, based on morphological characters.

Primitive characters of *S. nux-vomica*

Perennial ,long lived, leaves simple, net veined, flower bisexual, actinomorphic, hypogyny, regular, seed present, glabrous condition.

Advanced characters of *S. nux-vomica*

Opposite leaf arrangement, petals united, stamens differentiated into filament and anther, terminal stigma, carpels united, pollen triaperturate, cotyledons two, fruit a berry.

5.2 Phenological studies

Proper information on phenology provides an idea about life cycle and reproductive efforts that helps to prescribe conservation plan of a species as well as for formulating measures for cultivating such species on a large scale. *Nux-vomica* sheds

leaves during December-January and new flush emerges in February. Inflorescence bud burst in last week of February with peak blooming in between 15th March to 18th April. Fruits start developing during May, ripening during December to January. The phenophase observed in *nux-vomica* is common with other species of tropical dry deciduous forest (Singh and Shwaha 2006; Nanda *et al.*, 2014).

But the flowering pattern was asynchronous and has some significance also. Asynchronous flowering pattern is generally encountered in those species that require pollinator services especially insects, etc. for effecting allogamous pollinations and asynchronous flowering overcomes risk of pollinator limitations. Another important phenological feature observed in this species was the shy and alternating bearing flowering behaviour of its plants. Out of an approximate population size of about fifty plants growing at the study site, about four plants do not bore flowers and five to eight plants bloom alternatively and this trend was repeatedly observed for two seasons. This limits the possibility of good seed production that can be a source of genetic variation.

5.3 Pollen ovule ratio:

Pollen to ovule ratio (P/O) is considered to be an important floral trait that reflects directly towards the breeding system in angiosperm. Generally lowest P/O values correspond to obligate autogamous species while the highest correlate with obligate xenogamous species (Cruden 1977) though not universally applicable to all species. Number of seed production positively correlated with pollen limitation and subsequent resource limitation during fruit/seed development. The finding of higher P/O ratio of 335:1 in this species is an indicative of mixed mating system that is self as well as crossing (Affre *et al.*, 1995).

Like P/O ratio pollen load (the total number of pollen of the compatible flower on the stigma of another flower) also affects pollen competition and seed set. Several investigators have studied the degree of pollen load required for maximum seed set and it has been generalised that there should be at least 4 - 6 pollen grains per ovule on the stigma, to maximise seed set (Cruden 1977; Mulcahy *et al.*, 1983). Observations of this present work indicated that pollen load have a positive correlation with seed set percent. If all the twenty ovules of *nux vomica* are to become seed, an artificially applied pollen load of a minimum of approximately 200 pollen grains on stigma is

necessary. A lower pollen load from this the number of seeds produced per ovary might be lowered. Under xenogamy condition invariably 4.38 seeds per fruit (maximum 15 seeds per fruit) was produced in xenogamy as compared to 4.28 seeds per fruit in open pollination. Lower seed per fruit in autogamy (2.15 N/fruit) indicating that adequate pollen load is not available. Armstrong and Irvine (1989) reported a similar phenomenon in *Myristica insipida*.

5.3 Anther dehiscence and stigma receptivity

Stages of anther dehiscence and stigma receptivity vis-à-vis anthesis tell us about quantum of temporal synchrony or separation between male and female phase which in turn determines the breeding system of a species. While studying anther dehiscence in *S. nuxvomica* partial synchrony in anther maturity and stigma receptivity was observed which shows a mixed mating system i.e. both ‘homogamous and proterandrous’. Stigmas which are positioned at par or slightly above the anther level at the time of anther dehiscence become visibly receptive after 2.0 – 2.30 hours after shedding/ dehiscence of anthers. These stigmas slightly protrude out of corolla cavity due to increased stylar growth and also remain in receptive condition for another 10-12 hours, after complete anther shedding. The stigma receptivity could be visually observed by the changing colour pattern from cream yellow – crimson – black head (Fig.7). Mixed mating system (both self and cross pollination) have been reported in many tree species viz- *Juglans regia* L, *Madhuca longifolia*, *Emblia officinalis* etc. (Kumar et al., 2005; Aluri 1990).

5.4 Pollination studies and breeding behaviour

Accurate measure of prevailing breeding system in a plant species can be done through pollination studies. It helps in determining the degree to which an individual is self-fertilizing, outcrossing, apomictic or possesses a combination of these systems. Pollination studies also reveal whether the species is self and cross compatible. Breeding behaviour and reproductive biology studies are important to understand pollinator and plant interaction which is important for successful fertilization of any species and their survival. Critical observation on the study population made during two flowering seasons (2017 and 2018) revealed shy flowering as well as alternate

flowering habit. This flowering behaviour limits the chances of generating effective new variations.

In the present studies, a maximum fruit and seed set percent of 10.00 and 21.88 percent respectively was recorded as against only 9.775 and 21.25 percent fruit and seed set respectively in open pollinated flowers. In the flowers subjected to autogamy, least fruit and seed set of 7.15 and 10.75 percent respectively was observed as against 5.89 and 18.75 percent in geitonogamy. Based on fruit and seed set percentage, it is estimated that there are 17.94 to 39.74% chances of self pollination as compared to 29.78 to 60.26% chances of cross pollination. These above findings support the conclusion of Atluri *et al.* (2008) to the extent that *S. nuxvomica* favours both self as well as cross pollination. The higher seed set in cross pollination relative to self pollination indicates towards cryptic self incompatibility and in conformity with observations of Atluri *et al.* (2008). But detailed embryological study needed for confirmation. The mixed mating system i.e. simultaneous presence of cross and self pollen on the same stigma

5.5 Pollinators, floral visitors and their behaviour

Floral bud bursts in a sequential manner immediately after/ along with the new leaf flushing in strychnine tree. The flowers are not showy or attractive. The dark green colour of leaf at the time of blooming absorbs most of light falling on the tree and hence from a distance the cyme inflorescence is not distinctly visible. The aggregation of flowers in the terminal cyme makes the inflorescence more conspicuous. The odour of flower might act as a close –in attractant to entice landing of flower visitors. Anthesis takes place during evening hours and open flowers with nectar is available in dark hours but no flower visitors were observed. Probably the traces of nectar and odour might not be sufficient to attract nocturnal visitors.

Set of morphological and functional attributes of flower like color, odor, shape, reward, and anthesis etc. can be associated to pollination syndromes. These syndromes generally common or specific to plant species adapted to a certain type of pollinator (Martins and Batalha 2006). Pollination syndromes take into account these set of floral characters, which would allow determining the likeliest group of pollinators. The pollination syndrome of strychnine tree flowers includes pale corolla shape (tubular), colour (pale white colour of corolla), odour (mild sweet), and reward (pollen & honey). Though bees, flies, wasp, butter flies, beetles and birds observed visiting strychnine

tree flowers between 07.00 – 16.00 hours, it was the bees and butter flies are the regular visitors.

Insects were signalled by the flower scent and once they become closer to the tree, the colour combination (dark green leaf with pale white flower) attracts them towards flower. The tubular corolla allows bees and butter flies to land on the top of the flower. The bees probed the flowers in an upright position where the stigma head get easily contacted by virtue of its position and thus transfer of pollen load. Small size of corolla and position of longitudinally dehiscent anthers at the corolla brim makes easy access to rewards i.e. nectar and pollen. Pollination syndrome apart from attracting a number of flower visitors to forage flower but also insures their visiting rate sufficient for adequate pollination thus increasing the chances of out-crossing. This adoptive reproductive phenomenon of strychnine tree maintains the diversity of seed progeny.

5.5 Population structure

Quantitative analysis on tree species diversity provides an insight to the floristic status and distribution pattern in an ecosystem. Every species in nature adapts to specific habitat and have niche differentiation which enables it to utilize the resources to their maximum benefit. They bear some unique character and shows qualities such as dominance, diversity, stability and metabolic rate of their own. Study of these character like abundance, density, dominance (relative basal area), distribution pattern of a species in a habitat will help in judging the plant dynamics with changing environment and land use pattern.

S. nux-vomica is a member of tropical and sub-tropical forest biome and commonly located at the edges of dense forest, on river banks and along the shores. It is an emergent tree in mature and "undisturbed" forests and thought to be the transition stage of the succession between the pioneers and the "climax" forest (Behera *et al.*, 2017). Study of its natural occurrence in Chandaka-Dampara WL sanctuary confirms this fact. It was mostly observed in the open and partially opened places receiving ample sun light.

Tree density range 120 to 220 Nha⁻¹ within the protected area of sanctuary is in accordance with the observations of Panda (2017). Tree density range 20 to 70 Nha⁻¹ in fringes of sanctuary might be due to heavy disturbances and changing land use pattern. Reverse J-shaped GBH-density distribution curve were recorded for both the study sites

(within sanctuary and fringe area). According to several workers the reverse J-shaped population curve indicates climax or stable type of population in forest ecosystem (Kushwaha, & Nandy 2012.). The reverse J-shaped curve indicated heavy exploitation in higher girth classes in disturbed stand and less tree mortality and high density in lower girth classes in undisturbed stand (Rao *et al.*, 1990). Such types of population curve were reported in heavily disturbed forests of Eastern Ghats (Sahu and Mohanty 2012), and North-East India (Mishra and Tripathy 2005).

5.6 Natural regeneration

Natural regeneration in forest is a vital process for sylvigenesis of standing crop and assures long term stability. Successful management of a forest depends on good natural regeneration of dominant and valuable tree species (Halle *et al.*, 1978). Study of seedling dynamics i.e. changes in density and growth attributes in the matrix of disturbances assist in identifying the constraints hitherto suitable management practice. *S. nux-vomica* seed is one of the array of non-timber forest products supporting livelihood to thousands of tribals in Odisha. Hence study of regeneration status of this valuable species is essential for sustainable collection from forests.

Study of natural regeneration in turn requires a demographic assessment so as to understand the distribution pattern of different age/girth classes of a species in a community. Regeneration status of tree species can also be estimated by using dbh or gbh class distribution of trees or by understanding the structure and composition of their population. In this present study emphasis has been given to understand the impact of tree canopy and seed dispersal on seedling establishment and reproductive output of the *nux-vomica*.

Every organisms in nature adopts a unique type of regenerative strategy to remain fit in its community (Grime, 1979). Forest trees by and large have seed based reproductive strategies (*i. e.* by genets) though some species regenerates by ramets. In *kopilu* natural regeneration is primarily based on seed. Good seed year, proper seed dispersal, favourable environment for germination and growth of new recruits are necessary for ultimate establishment (Gomez-Pompa *et al.*, 1972).

Fruit maturity and seed dispersal in *kopilu* takes place during the spring season (February to early March) when forest floor is devoid of moisture. Seeds do not get adequate moisture for germination and also they have to escape long summer months

safely. Morphological feature of seed like hard seed coat protects embryo from excessive drying as well as physiological dormancy (Sivakumar *et al.*, 2006) to lie silently on the forest humus till monsoon drenches.

Seed mostly dispersed by gravity, water, frugivorous birds and animals. Seed germinating under the canopy spread area of mother trees survives less. It requires forest floor with good layer of humus for proper seed germination and open canopy area for seedling growth. This type of character is common with tropical forest tree species (Gadgil, 1971; Wyatt-Smith, 1963 and Unesco, 1978). The density of new recruits within (31.75 Nm^{-2}) and outside canopy spread area (8.15 Nm^{-2}) in the current year of seed fall and their survival (1.75 Nm^{-2} within & 0.64 Nm^{-2} outside mother tree canopy) and establishment (0 & 0.23 Nm^{-2}) after one year conforms to this fact.

CHAPTER VI

SUMMARY AND CONCLUSION

SUMMARY:

The present study has revealed that *S. nux-vomica* which is a common associate tree in tropical dry deciduous forest of Odisha is under stress due to ecological as well as biological reason. Long reproductive period (10-11 months) observed in its population impacts resource allocation for vegetative growth, flowering frequency etc. About 85-90 % of plants bloom in a asymmetric manner which favours outcrossing and generation of variability. Because of this variation nux-vomica is found to be best colonizer in seral stages of succession. As an aggressive adoptive feature, it produces numerous tricolpate pollen grains and has a mixed mating system which was ascertained by 29.78-60.26% cross pollination and 17.94 – 39.74% selfing. The higher pollen ovule ratio of 1700:1 per flower also supports to this observation.

As like other tropical tree species the regeneration under the canopy of mother tree maximum (31.75 Nm⁻² new recruits were observed) and their establishment after growth season reduces to zero. In contrast to this though the density of new recruits was only 8.15 Nm⁻² outside the mother tree canopy their establishment reduces to 0.23. The reverse J-shaped plant population structure both within the protected area of Chandaka-Dampara Wild life sanctuary as well as its fringes implies to the heavy disturbance and exploitation of higher girth classes.

CONCLUSIONS:

The brief conclusions that have emerged are as below:

Tree architecture:

1. Orthotropic nature of tree axis, crown type (columnar to oval), crown spread area (8.045 ± 0.703m), crown ratio percentage (76.82 ± 6.678) were reported for the first time.

Leaf:

1. Leaves are broad, opposite, simple, entire, smooth, short-stalked, blade orbicular to broadly elliptical or ovate, base rounded.
2. Leaf constants viz. Palisade ratio (5.61 ± 0.45), Vein-islet number ($49.64 \pm 4.36 / \mu\text{m}^2$), Vein termination number ($165.46 \pm 14.52 \mu\text{m}^2$), anomocytic type of stomata, Size of stomata ($94.635 \pm 8.025 \mu\text{m} \times 45.094 \pm 3.962 \mu\text{m}$), Stomatal index (16.97 ± 1.495) and stomata number (0.29 ± 0.03 per mm^2) have been recorded for the first time for this species.

Flower:

1. It is a shy and alternate bearing species. In the population 50 individuals (present study site) about 5-10% do not bear any flower and about 10-15% bears very sparsely.
2. Flowers are many, small in size, greenish-white colored, hermaphrodite, pentamerous actinomorphic, hypogynous, nectariferous and emits mild sweet odour.

Anthesis:

1. Anther dehiscence and stigma receptivity was observed to be partial homogamous and partial proterandus type (mixed type). The capitates stigma becomes receptive after 2.0-2.30 hours of anthesis and retains its receptivity 8-12 hours after completion of pollen dehiscence and withering of stamens. This phenomenon was not reported earlier.
2. Pollen was tricolpate & Pollen ovule ratio was determined to be 335:1 per flower, indicating preference for cross pollination.
3. 99.36% fresh pollen was viable and gradually reduces viability after 72 hours of dehiscence (10.08%).

Pollination:

1. The breeding system is of mixed type. It favours cross pollination (29.78-60.26%) however selfing is also possible (17.94 – 39.74%).
2. Fruit set percentage was 10.00% in xenogamy, 9.775% in open pollination, 7.15% in autogamy and 5.89% in geitonogamy.
3. Seed set % was maximum 21.88% in xenogamy and minimum 10.75% in autogamy.
4. Seed viability was cent percent in cross pollination and 95% in selfing.
5. Bees (*Apis dorsata*) and butter flies were the principal pollinators
6. Seed per fruit varies with size and ranges from 1 to 14 as reported earlier 1 – 5

Population structure:

1. Reverse J-shaped population curve was observed indicating heavy disturbance in its natural habitat and heavy exploitation in higher girth classes.

Natural regeneration:

1. Density of new recruits reduces with increasing distance from the mother tree (35.75 to 1.52 Nm^{-2}). Whereas density of established regeneration (>2.0 m height) increases with distance (0 to 0.23 Nm^{-2}).

Recommendations

1. *S. nux-vomica* favours cross pollination and bees are the principal pollinators. Hence maintenance of pollinator population in plantation will ensure maximum fruit and seed yield.
2. Conservation of higher classes is necessary for a viable population in nature.
3. Canopy management ensuring open space for better natural regeneration.

Future thrust of research

1. Evaluation of elite genotypes possessing higher percentage of alkaloids, heavy & regular fruiting capacity.
2. Standardization of vegetative method of propagation.
3. Standard package and practice of cultivation.

REFERENCES

1. Affre L., Thompson, J. D. and Debussche, M. 1995. The Reproductive biology of Mediterranean endemic *Cyclamen balearicum* Willk (Pnmulaceae). *Bot J Linn Soc.* 118 309-330
2. Akbar, S., Khan, S. A., Masood, A. and Iqbal, M. 2010. Use of *Strychnos nuxvomica* (Azraqi) seeds in Unani system of medicine: role of detoxification. *Afr J Tradit Complement Altern Med.* 7(4):286-290
3. Aluri, R. J. S. 1990. Studies on pollination ecology in India. *Proc. Indian Natn. Acad.* B56 (4):375-388
4. Anonymous, 1999. The Ayurvedic Pharmacopoeia of India. Government of India, NISCAIR, New Delhi. 1(4): 158-160 (<http://www.ayurveda.hu/api/API-Vol-4.pdf>)
5. Anonymous, 2008. Agro-techniques of selected medicinal plants. National Medicinal Plants Board, Department of AYUSH, Ministry of Health and Family Welfare, Government of India. Vol. 2:179-182.
6. Ansari, A. A., 1989. Phenological aspect of Madhauria forest, Gorakhpur. *Indian Forester.* 115: 252-257
7. Arivoli, S., and Tennyson, S. 2012. Larvicidal Efficacy of *Strychnos nuxvomica* Linn. (Loganiaceae) leaf extracts against the filarial vector *Culex quinquefasciatus* say (Diptera: Culicidae). *World Journal of Zoology.* 7(1): 06-11
8. Arjunan, M. C., Selvi, M. J. and Lakshmanan, K. K., 1995. Phenology of some woody angiosperms of Coimbatore district. *Annals of Forestry.* 3(1): 45-52
9. Armstrong, J. E and Irvine, A. K. 1989 Flowering, sex ratios, pollen-ovule ratios, fruit set, and reproductive effort of a dioecious tree, *Mynstica insipida* (Myristicaceae), in two different rain forest communities. *Am. J. Bot.* 76 : 74-85
10. Ashraf, M., Ayub, M., Sajjad, T., Elahi, N., Ali, I., Ahmed, Z. 2010. Replacement of rotenone by locally grown herbal extracts. *Int. J. Agric. Biol.* 12:77–80
11. Atluri, J. B., Ramana, S. P. V. and Subba Reddi, C. 2008. Eventide blooming, Insect pollination, low fruiting and seeding in the strychnine tree, *Strychnos nuxvomica* Linn. *Proc. Nat. Acad. Sci. India, Sect. B.* 78(1):66-71

12. Barthelemy, D. and Caraglio, Y. 2007. Plant architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Annals of Botany*. 99: 375–407
13. Behera, M. C., Mohanty, T. L. and Paramanik, B. K 2017. Silvics, phytochemistry and ethnopharmacy of endangered poison nut tree (*Strychnos nux-vomica* L.): A review. *Journal of Pharmacognosy and Phytochemistry*. 6(5): 1207-1216
14. Beniwal, B. S., 1987. Phenological study of trees in Arunachal Pradesh. *Indian Forester* **113**: 779-791
15. Bhat, D. M., Murali, K. S., Ravindranath, N. H. 2001. Assessment of propagation techniques for forest species of the Western Ghats region of India. *Forests, Trees and Livelihoods*. 11(3):233-250
16. Bhati, R., Singh, A., Saharan, V. A., Ram, V., Bhandari, A. 2012. *Strychnos nux-vomica* seeds: Pharmacognostical standardization, extraction, and antidiabetic activity. *Journal of Ayurveda & Integrative Medicine*. 3(2):80-84
17. Bhuyan, P., Khan, M. L. and Tripathi, R. S. 2003. Tree diversity and population structure in undisturbed and human - impacted stands of tropical wet evergreen forest in Arunachal Pradesh, Eastern Himalayas, India. *Biodiver. Conserv.* 12: 1753-1773
18. Biala, R. G., Tits, M., Penelle, J., Federich, M., Brandt, V., Prosperi, C., Liabres, G. And Angenot, L. 1998. Strychnochrysine a new bisindole alkaloid from the roots of *Strychnos nux-vomica*. *J. Nat. Prod.* 61(1):139–141
19. Bodeker, G. C. 1997. Introduction. **In**: Food and Agriculture Organization: medicinal plants for forest conservation and health care. Food and Agriculture Organization, Rome, Italy. Pp.1–4.
20. Boso, S., Gago, P., Alonso-Villaverde, V., Santiago, J. L. and Martinez, M. C. 2016. Density and size of stomata in the leaves of different hybrids (*Vitis* sp.) and *Vitis vinifera* varieties. *Vitis* 55:17–22
21. Cai, B. C. Yang, X. W., Hattori, M., Nainba, T. 1994. Anaysis of spectral data for BC NMR of sixteen *Strychnos* alkaloids. *Acta Pharmaceutica Sinica*. 29 (1): 44-48
22. Chacko, V. J. 1965. A manual of sampling techniques for forest surveys. Manager publications, Delhi. 172p.
23. Chaubey, O. P. and Krishnamurthy, G. 2015. Biotechnological approach of threatened species *Strychnos nux-vomica* L. to standardized nursery techniques. *International Journal of Bio-Science and Bio-Technology*. 7(6):13-24

24. Chitra, V., Varma, P. V., Raju, M. K., Prakash, K. J. 2010. Study of antidiabetic and free radical scavenging activity of the seed extract of *Strychnos nuxvomica*. *International Journal of Pharmacy and Pharmaceutical Sciences*. Vol-2 (Suppl-1): 106-110
25. Chopra, R. N., Chopra, I. C., Handa, K. L. and Kapur, L. D. 2006. Indigenous drugs of India. Academic Publisher, Kolkata. Pp-248-250
26. Cronquist, A. 1968. The evolution and classification of flowering plants. Boston : Houghton Mifflin, pp. 143-145
27. Cruden, R. W. 1977. Pollen ovule ratio: a conservative indicator of breeding system in flowering plants. *Evolution*. **31**(1):32–46
28. Cruden, R.W. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution*. **31**: 32–46
29. Dafni, A. 1992. Pollination ecology: a practical approach. New York. Oxford University Press, New York
30. Dafni, A., Pacini, E. and Nepi, M. 2005. Pollen and stigma biology. In: Dafni A., Keven P. G. And Husband B. C. Practical pollination biology. Ontario Enviroquest. 83-146
31. De, B. and Datta, P. C. 1988. Alkaloids of *Strychnos nux-vomica* flower. *Planta Medica*. **54** (4):363
32. Eldahshan, O. A., Abdel-Daim, M. M. 2015. Phytochemical study, cytotoxic, analgesic, antipyretic and anti-inflammatory activities of *Strychnos nux-vomica*. *Cytotechnology*. **67**:831–844
33. Evens, W. C. 2009. Trease and Evans Pharmacognosy (16th Edition). 399-400
34. Fahn, A. 1967. Plant anatomy. Oxford : Pergamon Press, pp. 162-240
35. Frederich, M., Choi, Y. H., Angenot, L., Harnischfeger, G., Lefeber, A. W. M. and Verpoorte R. 2004. Metabolomic analysis of *Strychnos nux-vomica*, *Strychnos icaja* and *Strychnos ignatii* extracts by ¹H nuclear magnetic resonance spectrometry and multivariate analysis techniques. *Phytochemistry*. **65** :1993–2001
36. Gadella, T. W. J. 1966. Some cytological observations in the Loganiaceae III. *Acta Bot. Neerl.*, **15**: 490-491
37. Gadgil, M. 1971. Dispersal: population consequences and evolution. *Ecology* **52**: 253-261
38. Gomez, K. A. and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research (2nd ed.), John Wiley and Sons Inc, New York – USA. 680p

39. Gomez-Pompa, A., Vazquez-Yanes, C. and Guevara, S . 1972. The tropical rain forest: a non-renewable resource. *Science* 177: 762-765
40. Good, N. F. and Good, R. E. 1972. Population dynamics of tree seedlings and saplings in mature eastern hardwood forests. *Bull. Torrey Bot. Club.*, 99:172-178
41. Grime, J. P. 1979. Regenerative strategies. Pages 79-122. In: Grime, J. P. (ed.). *Plant strategies and vegetation process*. John Wiley and Sons, New York
42. Grubb, P. J. 1977. The maintenance of species richness in plant communities: The importance of regeneration niche. *Biological Reviews*. 52:107-145
43. Gupta, R. C. 2012. (Ed). Veterinary toxicology Basic and clinical principles. Academic Press. USA. 698-700
44. Haines, H. H. 1961. The botany of Bihar and Orissa. Gouranga Press Private Ltd . Kolkata. Vol-2: 592
45. Halle, F., Oldeman, R. A. A. and Tomlinson, P. B. 1978. Tropical trees and forests: an architectural analysis. Springer - Verl ag, Berlin
46. Hill, A. W. 1917. The Genus *Strychnos* in India and the East. Bulletin of Miscellaneous Information, Royal Botanic Garden, KEW. 4/5:121-210
47. Jindal, S. K., Solanki, K. R., and Kalker, N. L., 1985. Phenology and breeding system of Rohida (*Tecomella undulate* Seem.). *Indian Journal of Forestry*. 8(4): 317-320
48. Jonville, M. C., Dive, G., Angenot, L., Bero, J., Tits, M., Olliver, E., Federich, M. 2013. Dimeric bisindole alkaloids from the stem bark of *Strychnos nux-vomica* L. *Phytochemistry*. 87:157–163
49. Joy, P. P., Thomas, J., Mathew, S., Skaria, B. P. 1998. Medicinal Plants, Kerala Agricultural University, Aromatic and Medicinal Plants Research Station. 164-165
50. Katiyar, C., Kumar, A., Bhattacharya, S. K., Singh, R.S. 2010. Ayurvedic processed seeds of *nux-vomica*: Neuropharmacological and chemical evaluation. 81(3): 190-195
51. Kaufman, P. B.; Carlson, T. F.; Dayanandan, P.; Evans, M. L.; Fisher, J. B.; Parks, C. and Weils, J. R. 1989. Plants their biology and importance. New York: Hopper and Raw Publishers. 532: 714-730

52. Khan, M. L., Rai, J. P. N. and Tripathi, R. S. 1987. Population structure of some tree species in disturbed and protected sub-tropical forests of NE India. *Acta Oecol.*, 8: 247-255
53. Kirtikar, K. R., Basu, B. D. 1991. Indian Medicinal Plants. Vol. 3. Bishen Singh Mahendra pal Singh Publisher: Dehradun, India. 629-630
54. Kukade, S. A. and Tidke, J. A. 2013. Studies on pollination and reproductive biology of *pongamia pinnata* l. (Fabaceae). *Indian Journal of Fundamental and Applied Life Sciences*. Vol. 3 (1):pp.149-155
55. Kumar, A and Datta, S. K. 1989. Plantlet regeneration from hypocotyl tissue of *Strychnos nux-vomica* Linn. *Current Science*. 58(14):812-813
56. Kumar, A. 1990. Regeneration of forest tree *Strychnos nux vomica* L. through tissue culture. *Indian Journal of Forestry*. 13(3): 263-265
57. Kumar, K., Sharma, R. and Sharma, S. D. 2005. Homogamy in Persian walnut (*Juglans regia* L.) selections of indigenous origin from Himachal Pradesh, India. *Advances in Horticultural Science* 19(1):29-33
58. Kushwaha, S. P. S. and Nandy, S. 2012. Species diversity and community structure in sal (*Shorea robusta*) forests of two different rainfall regimes in West Bangal, India, *Biodiversity and Conservation* 21 (5):1215-1228
59. Lawrence, G. H. M. 1951. Taxonomy of vascular plants. McMillan, New York.
60. Leela, N. K., Pervez, R., Ramana, K. V., Rosana, O. B. and Eapen, S. J. 2012. Nematicidal activity of *Strychnos nuxvomica* leaf and its constituents against the root-knot nematode, *Meloidogyne incognita*. *Nematol. medit.* 40:157-162.
61. Ma, L., Gu, R., Tang, L., Chen, Z. E., Di, R., Long, C. 2015. Important poisonous plants in tibetan ethnomedicine. *Toxins*. 7:138-155
62. Maji, A. K., Banerji, P. 2017. *Strychnos nux-vomica*: A poisonous plant with various aspects of therapeutic significance. *J Basic Clin Pharma*. 8:S087-S103
63. Martins, F. Q. and Batalha, M. A. 2006. Pollination systems and floral traits in cerrado woody species of the upper taquari region (Central Brazil). *Braz. J. Biol.*, 66(2A):543-552
64. Mishra, B. P., Tripathy, O. P. and Laloo R. C. 2005. Community characteristics of a climax subtropical humid forest of Meghalaya and population structure of ten important tree species, *Tropical Ecology*. 46 (2):241-251

65. Misra, R. 1968. Ecology workbook. Oxford & IBH Publishing Company. Delhi
66. Mitesh, C., Kalpana, C., Shama, S. 2015. Upavisha kuchala (*Strychnos nuxvomica* Linn.) – a review. *International Ayurvedic Medical Journal*, 3(7): 2139-2147
67. Mitra, S., Acharya, A. 2012. A comprehensive review on therapeutic utilities and purificatory procedures of *Strychnos nux-vomica* Linn. (Kupeelu) as described in Indian systems of medicine. *Journal of Ayush*.1(1):1-8
68. Mohrbutter, C. 1936. Embryologische Studien an Loganiaceen. *Planta*, 26:64-80
69. Mulcahy, D. L., Curtis, P. S. and Snow, A. A. 1983. Pollen competition in a natural population: In Handbook of Experimental Pollination Biology (eds), C E Jones and R J Little Van Nostrand Remhold, New York pp 330-337
70. Murali, K. S. 1992. Vegetative and reproductive phenology of a tropical dry deciduous forest, Southern India. Ph.D. Thesis. Submitted to Centre for Ecological Conservation, Indian Institute of Sciences, Bangalore
71. Murali, K. S. and Sukumar, R. 1994. Reproductive phenology of tropical dry forest in Mudumalai, South India. *The Journal of ecology*. 82(4):759-767
72. Nadkarni, K. M. 1954. Indian Materia Medica. Popular Book Depot. Vol-1:1175-1181.
73. Nanda, A., Suresh, H. S and Krishnamurthy, Y. L. 2014. Phenology of a tropical dry deciduous forest of Bhadra wildlife sanctuary, southern India. *Ecological Processes*. 3:1-12
74. Newstrom, L. E., Franki, G. W and Baker, H. G. 1994. A new classification for plant phenology based on flowering patterns in lowland tropical rain forest trees at La Selva. Costa Rica. *Biotropica*, 26:141-159
75. Panda, P. C. 2017. Qualitative and quantitative assessment of plant diversity of Chandaka-Damapara Sanctuary with special emphasis on identification of keystone, rare and useful species and their habitats. Final Technical Report. Chandaka Wildlife Division, Bhubaneswar.
76. Patel, D. K., Patel, K., Duraiswamy, B. and Dhanabal, S. P. 2012. Phytochemical analysis and standardization of *Strychnos nux-vomica* extract through HPTLC techniques. *Asian Pacific Journal of Tropical Disease*. 2(Suppl-1):S56-S60

77. Patel, K., Laloo, D., Singh, G. K., Gadewar, M., Patel, D. K. 2017. A review on medicinal uses, analytical techniques and pharmacological activities of *Strychnos nux-vomica* Linn.: A concise report. *Chinese Journal of Integrative Medicine*. 2017; 1-13
78. Prakasha, H. M., Krishnappa, M., Krishnamurthy, Y. L and Poornima, S. V. 2010. Folk medicine of NR Pura Taluk in Chikamagalur district of Karnatka. *Indian Journal of Traditional Knowledge* 9(1):55–60
79. Prasad, M. N. V., Padmalatha, K., Yayaram, K., Raju, N. L. and Silva, J. A. T. 2007. Medicinal plants from Deccan Ecoregion, India: Traditional knowledge, ethnopharmacology, cultivation, utilization, conservation and biotechnology-opportunities and impediments. *Medicinal and Aromatic Plant Science and Biotechnology*. 1(2): 155-208
80. Pulliah, T., Rani, S. S. 2011. Flora of Eastern Ghats, Regency Publication. New Delhi. India. 4:135-139
81. Raju, A. J. S and Ezradanam, V. 2002. Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (Euphorbiaceae); *Current Science*, VOL. 83(11): pp.1395-1398.
82. Raju, A. J. S, Lakshmi, P. V. and Ramana, K. V. 2012. Reproductive ecology of *Terminalia pallida* Brandis (Combretaceae), an endemic and medicinal tree species of India. *Current Science*; 102(6). PP-909-917
83. Raju, A. J. S. and Rao, S. P. 2002. Pollination ecology and fruiting behaviour in *Acacia sinuata* (Lour.) Merr. (Mimosaceae), a valuable non-timber forest plant species; *Current Science*, VOL. 82(12): pp1466-1471
84. Raju, A. J. S., Krishna, J. R. and Chandra. P. H. 2014. Reproductive ecology of *Syzygium alternifolium* (Myrtaceae), an endemic and endangered tropical tree species in the southern Eastern Ghats of India; *Journal of Threatened Taxa*. 6(9): 6153–6171
85. Ralhan, P. K., Khanna R. K., Singh S. P., and Singh J. S. 1985. Phenological characteristics of tree layer of Kumaun Himalayan forests. *Vegetation*, 60 (2): 91-101

86. Rao, P., Barik, S. K., Pandey, H. N., Tripathi, R. S. 1990. Community composition and tree population structure in a sub-tropical broad-leaved forest along a disturbance gradient. *Vegetation* 88: 151-162
87. Rao, S. P. and Raju, A. J. S. 2002. Pollination ecology of the red sander (*Pterocarpus santalinus*; Fabaceae) an endemic and endangered tree species. *Current Science*. 83(9): 1144-1148
88. Reddi, S. C., Reddi, E. U. B., Reddi, N. S. and Reddi, P. S. 1983. Reproductive ecology of *Sapindius emarginatus* Vahl. (Sapindaceae). *Proc. Indian national Science Academy*. 49 B (1):57-72
89. Sahu, S. C., Dhal N. K. and Mohanty, R. C. 2012. Tree species diversity, distribution and population structure in a tropical dry deciduous forest of Malyagiri hill ranges, Eastern Ghats, India, *Tropical Ecology*. 53 (2): 163-168
90. Sankar, V. R., Ravikumar, K. and Goraya, G. S. 2008. Floristic wealth of Javvadhu Hills, Eastern Ghats, With Special Emphasis on Threatened Plants. In: Special Habitats and Threatened Plants of India, Envis Bulletin. Wildlife Institute of India. 11(1): Pp-195-201
91. Schmelzer, G. H., Gurib-Fakim, A. 2008 (Ed.) Plant Resources of Tropical Africa (PROTA), Medicinal Plants-1, Backhuys Publishers, Wageningen, Netherlands.; 11(1):575-577
92. Shi, Y., Liu, Y., Ma, S, Qu, L. L. J., Li, Y. and Yu, S. 2014. Four new minor alkaloids from the seeds of *Strychnos nux-vomica*. *Tetrahedron Letters*. , 55:6538–6542
93. Shi-Jin, L. Dian-Xiang, Z., Lin, L. and Zhong-Yi C. 2004. Pollination ecology of *Caesalpinia crista* (Leguminosae: Caesalpinioideae). *Acta Botanica Sinica*, 46 (3): 271-278
94. Shiva, M. P. 1998. Inventory of Forest Resources for sustainable management and biodiversity conservation, Indus Publishing Company, New Delhi, India. 196-197
95. Shivaprasad, D., Prasannakumar, C. N., Somashekar, R. K. and Nagaraja, B. C. 2015. Reproductive biology of *Cinnamomum sulphuratum* Nees. from wet evergreen forest of Western Ghats in Karnataka. *Proceedings of the International Academy of Ecology and Environmental Sciences*; 5(1): 7-15
96. Singh, K. P. and Shwaha, C. P. K. 2006. Diversity of flowering and fruiting phenology of trees in a tropical deciduous forest in India. *Annals of Botany*. 97: 265–276

97. Sivakumar, V, Anandalakshmi, R, Warriar, R. R., Tigabu, M. Oden, P. C, Vijayachandran, S. N, Geetha, S. and Singh, B. G. 2006. Effects of presowing treatments, desiccation and storage conditions on germination of *Strychnos nux-vomica* seeds, a valuable medicinal plant. *New Forests*.32:121–131
98. Sivaraman, G., Paulraj, M.G, Ignacimuthu, S, Al-Dhabi, N. A. 2014. Bioefficacy of seed extracts of *Strychnos nux-vomica* and *Semicarpus anacardium* against *Helicoverpa armigera* (Hubnar) (Lepidoptera: Noctuidae). *International Journal of Agricultural and Food Science*. 4(2):73-77
99. Smitha, G. R and Thondaiman, V. 2016. Reproductive biology and breeding system of *Saraca asoca* (Roxb.) De Wilde: a vulnerable medicinal plant. *Springer Plus*; 5: PP-1-16
100. Solomon, Raju. A. J. and Rajesh, B. 2014. Pollination ecology of Chengam *Scyphiphora hydrophyllacea* C.F. Gaertn. (Magnoliopsida: Rubiales: Rubiaceae) a non-viviparous ever green tree species. *Journal of Threatened Taxa*. 6(14):6668-6676
101. Somashekhar, B. S., Sharma, M. 2002. Training manual on propagation techniques of commercially important medicinal plants. Andhra Pradesh State Forest Department. 110-111
102. Somashekhar, B. S., Sharma, M. 2002. Training manual on propagation techniques of commercially important medicinal plants. Andhra Pradesh State Forest Department. 110-111.
103. Soosairaj, S., Kala, A., Raja, P. and Balaguru, B. 2014. Reproductive biology of the tropical vulnerable shrub *Capparis shevaroyensis* Sund. –ragh. (Capparaceae). *Life Sciences Leaflets*. 52:71-78
104. Sreekala, A. K., Pandurangan, A. G., Ramasubbu, R. and Kulloli, S. K. 2011. Pollination biology of *Imptiens cuspidate* Wight and Arn (Balsaminaceae) a rare and endemic balsam of the Western Ghats, India. *Journal of Threatened Taxa*. 3(6):1818-1825
105. Subbaiah, K. P. V., Savithramma, N. 2014. Validation and characterization of silver nanoparticles from *Strychnos nux-vomica*– an important ethnomedicinal plant of Kurnool district, Andhra Pradesh, India. *International Journal of Pharmacy and Biological Sciences*. 4(1):45-53.

106. Thambi, M., Cherian, T. 2015a. Phytochemical investigation of the bark of *Strychnos nux-vomica* and its antimicrobial properties. *The Pharma Innovation Journal*. 4(5):70-72
107. Thambi, M., Cherian, T. 2015b. Pesticidal Properties on the Leaf Extracts of *Strychnos-Nux-Vomica* Plant. *Journal of Innovations in Pharmaceuticals and Biological Sciences*. 2(3):266-272
108. Turner, T. D., Davies, J. M. 1963. The morphology and histology of the seeds of *Strychnos nux-vomica*, Linn. and its adulterants *Strychnos nux-blanda* Hill, and *Strychnos potatorum*, Linn. *Journal of Pharmacy and Pharmacology*. 15(1):594-610
109. Unesco. 1978. Chapter 8. The natural forest: plant biology, regeneration and tree growth. In: *Tropical forest ecosystems*: 180-215. Unesco-UNEP, France.
110. Vanangamudi, K., Natarajan, K., Saravanan, T., Natarajan, N., Umarani, R., Bharathi, A. and Srimathi, P. 2006. Advances in seed science and Technology. 3:162-163
111. Victor, A. D. D., Prasad, M. P. N. and Mohana, S. S. 2016. Biological active compounds with various medicinal values of *Strychnos nux-vomica*- A Pharmacological summary. *J Global Trends Pharm Sci*. 7(1): 3044-3047.
112. Weberling, F. 1989. Morphology of flower and inflorescence. Cambridge University Press, New York.
113. Wyatt-Smith, J. 1963. Manual of Malayan silviculture for inland forests. *Malay. For. Rec. no. 23*
114. Yang, G., Tu, X., and Liu, L., Pan, Y. 2010. Two new bisindole alkaloids from the seeds of *Strychnos nux-vomica*. *Fitoterapia*. 81:932–936.
115. Zhang, J. Y., Li, N., Hu, K. and Tu, F. 2012. Chemical constituents from processed seeds of *Strychnos nux-vomica*. *Journal of Chinese Pharmaceutical Sciences*. 21:187–191.
116. Zhao, N., Li, L., Liu, J. Zhuang, P., Yu, S., Ma, S., Qu, J., Chen, N. and Wu, L. 2012. New alkaloids from the seeds of *Strychnos nux-vomica*. *Tetrahedron*. 68 (16):3288-3294.
117. Saxena, H. O and Brahmam, M. 1994. The flora of Odisha. Orissa Forest Development Corporation Ltd. Pp-1113-11