CHAPTER-2

MATERIALS AND METHODS

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The present dissertation is cantered around an endemic and critically endangered Asclepiad and its habitat. The extremely narrow distribution pattern and availability of this herbland (more preferably, grassland) species arouse natural interest in mind, specially about its habitat structure and pattern of reproduction and/or propagation.

2.1 MORPHOLOGICAL STRUCTURES OF *Streptocaulon sylvestre* WIGHT

For better understanding of the taxon its various morphological structures have been studied in detail and is presented below:

Streptocaulon sylvestre Wight, Contrib. Ind. Bot. 65.1834; Hook.f., Fl. Brit. Ind. 4:10. 1883; Prain Beng. Pl. 2:509 (1903); Das in J. Bomb. Nat. Hist. Soc. 93(2): 320 - 322. 1996.

A suffrutescent strictly procumbent herb with milky latex, never rooting from branches; stem terete, c. 0.13 cm in diam, mature internodes 1.5 – 4.0 cm, shortly tomentose, remotely branched, forms a close carpet when grow in open condition. Roots very deep-seated, sometimes more than a metre long, around 0.7 cm in diam., with very few slender lateral branches. Leaves opposite, with 0.3 - 0.4 cm long petiole; lamina (4- 6.4 x 3.7 - 5.9 cm) ovate to rounded-ovate, entire, apiculate, shortly cordate-auriculate, leathery, shortly hispid below, minutely pubescent above; 5-vained from base, mid-vein strong, 5-7 nerved laterally, gradually curved upwards, all veins elevated below. Bracts linear-subulate, 0.15- 0.22 cm long, green, hairy. Flowers in axillary, shortly peduncled (0.5 - 0.8 cm), 1 - 9 flowered cymes, shortly pedicellate (0.5 - 1.1 cm), actinomorphic, hypogynous, bisexual, 1 - 1.2 cm in diam. Sepals 5, 0.2-0.25 x 0.08-0.09 cm, connate only at base, broadly ovate, entire, acute, hairy, quincuntial, brownish-purple, alternating with linear glands at base, slightly enlarged in fruit. Corolla rotate with a very short (0.1 cm) greenish- white tube; lobes 5, 0.7 - 0.8 x 0.15 cm, linear oblong, obtuse, twisted to the right, curved downward after opening, deep purple. Stamens 5, inserted on the base of corolla, antipetalous; filaments short (0.04-0.1 cm), flattened, white, alternating with minute teeth; anthers flattened, 0.1 -0.11 cm, oblong, bithecal, base slightly sagittate, with a short triangular corona, conniving and attached near the tip of stigma; pollen masses

2 in each cell, linear, attached to a slender and short corpuscle, tip dilated; corona produced from the base of filament, slender whip-like, 0.6-0.65 cm long, deep brown at base, white above, tips do not coil. Carpels 2, united little below the stigma; ovaries 2, oblong, 0.7-0.75 cm long, hairy, 1-chambered each, ovules numerous, inserted on marginal placenta; style short; stigma conical, cap like margin 5-lobed, each lobe cordate, obscurely 2-lobed at tip. One or a pair of follicles generally develop, terete with a longitudinal ventral furrow, nearly oblong to elliptic, tapering from middle to tip, slightly narrowed towards the base, smooth walled, minutely villous, 2.5 - 8.8 × 1-1.3 cm, dehiscent; seeds numerous, oblong - elliptic to ovate, flat, brown to reddish brown; hairs of coma 1 - 4.4 cm long, white, lustrous, spreading widely when open. Embryo straight, surrounded by a very thin and transparent layer of endosperm; cotyledons ovate-elliptic, fleshy, white, with no prominent nerve; radicle short and conical.

Entire population, so far known to us is almost uniform in vegetative characters. New leaves produced on sprouting during late winter (i.e. February – March) looks quite different (nearly ovate, acute or even slightly acuminate) which on maturity, gradually, change its shape to almost a rounded structure. Leaves produced later do not show such modifications and remain rounded-ovate to rounded from the beginning. The size of lamina, however, is according to health of the plant and also on the nature of exposure to sun. The number of flowers per inflorescence is uniform in the population, but it varies in different seasons of the year. A maximum number of flowers per inflorescence (i.e. 09) are available during late winter and the number of flowers decreases with the increase of ambient temperature. The optimum flowering period is during March – April. Also, there is no any significant variation in the size of flowers or its different parts except that winter flowers are slightly larger.

In fruits, however, there are significant differences. There are clearly three different sizes of fruits, which may be recognised as

(i) *long fruit*,
(ii) *medium fruit* and
(iii) *small fruit*.

Seeds in these three categories of fruits are also different in shapes and sizes. Differences between these three types of fruits are presented here in Table 2.1.

In future, it may be possible to break these three fruit-forms into infraspecific taxa for which further studies are essential to confirm such action.

Table 2.1: Comparison of three size classes of fruits of *Streptocaulon sylvestre* Wight

Characters	Fruit size		
	Large	Medium	Small
Shape	Nearly oblong, tapering from middle to tip, slightly narrowed towards the base	Oblong, tapering from middle to tip, slightly narrowed towards the base	Oblong to elliptic, tapering from middle to tip, narrowed towards the base
Length cm	7.78 ± 0.45	6.00 ± 0.11	3.59 ± 0.16
Circumference at broadest part (cm)	3.12 ± 0.11	3.82 ± 0.07	2.84 ± 0.15
Fruits per plant	3.80 ± 0.37	4.60 ± 1.12	5.80 ± 1.74
Maturity time	170 - 190 days	170 - 190 days	170 - 190 days
No. of seeds per fruit	32.47 ± 2.35	41.73 ± 4.23	20.65 ± 1.47
Size of produced seeds	Large	Large & Medium	Medium & Small

During May-June, when seedlings appear, sometimes freaks with 3-4 cotyledons are also observed. These seedlings, however, do not maintain this condition in normal nodes. When a large number of seedlings are developed on a pot, in rare cases, seedlings with alternate phyllotaxy are also found, which also gradually revert back to its normal opposite-decussate condition.

SPECIMEN CITED : North Bengal University campus, A.P. DAS & A.F.M.M. Kadir 005, April 21, 1996 (NBU-Herbarium)

2.2. PHYTOSOCIOLOGY

2.2.1. SAMPLING OF THE VEGETATION

The phytosociological analysis of four selected herblands was performed by quadrat method of sampling. As all the selected vegetations were small and herbaceous,

a standard quadrat size of 1m × 1m was selected to use. However, Misra (1968) was followed for the determination of size and number of quadrats. The study was conducted during July 1997 to June 1999 for Site-I and Site-II (North Bengal University Campus plot-1 & 2) and July 1998 to June 1999 for Site-III (Sanyasikata) and Site-IV (Kharibari). On the basis of climate, the year was divided into three distinct seasons namely, Monsoon (mid June to October), Winter (mid-October to March) and a short Summer (April to mid June). 20 quadrats were laid down randomly at each Site in each season during the survey period (i.e. a total of 360 quadrats for four different Sites). All the sites left unprotected i.e. without any fensing to realise the situation what is going on in the vegetation.

Data for list, count and basal cover of all the available species in quadrats were recorded in the Field Note Book. The field records also included the habit and habitat, abundance, life form, flowering and fruiting period, flower colour, odour and such characters of plants which are not available with dry herbarium specimens.

2.2.2. COLLECTION AND PRESERVATION OF MATERIALS

Representative specimens were collected with leaves, flowers and / or fruits (if available) from sample points (i.e. from quadrats), which were tagged and kept temporarily in polythene bags. Collected specimens were brought to the laboratory in such condition and were then separated, cleaned and properly displayed on the blotting papers and finally inserted in a heavy Wooden Plant Press for drying. Blotting papers were changed several times. Sometimes specimens were treated with formaldehyde for better preservation. During monsoon season, for quick drying a hot air oven was also used.

However, after proper drying, all specimens were poisoned by soaking with 8% solution of Mercuric Chloride in rectified spirit and dried again under pressure using blotting papers. Dried and poisoned specimens were then pasted on standard herbarium sheets and labelled with all relevant informations. Loose floral parts, flower, small fruits and /or seeds as well as different parts were placed in paper packets or envelops which were, in tern, affixed to the herbarium sheets.

2.2.3. IDENTIFICATION

Identification of collected specimens were primarily done at the Plant Taxonomy Laboratory of North Bengal University, using literature and was then matched and confirmed at the NBU-Herbarium and at the Central National Herbarium

(CAL.), Howrah. After the conclusion of the dissertation, the Field Note Book and Specimens will be deposited in the NBU- Herbarium.

2.2.4. DATA ANALYSIS

Using list, count and basal cover data of quadrats following phytosociological parameters were calculated.

2.2.4.1 ABUNDANCE, DENSITY, FREQUENCY, RELATIVE DENSTIY, RELATIVE FREQUENCY, RELATIVE DOMINANCE AND IMPORTANCE VALUE INDEX OF EACH SPECIES OF HERBLANDS.

All these parameters were determined as suggested by Misra (1968) and Phillips (1959).

$$\textbf{Abundance (Ab)} = \frac{\text{Total number of plants of a species in all the quadrats}}{\text{Number of quadrats in which the species occurred}}$$

$$\textbf{Density (D)} = \frac{\text{Total number of individuals of a species in all the quadrats}}{\text{Number of quadrats examined}} \text{ plant m}^{-2}$$

$$\textbf{\% Frequency (F)} = \frac{\text{Number of quadrats in which the species occurred}}{\text{Total number of quadrats examined}} \times 100$$

$$\textbf{Relative Density (RD)} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Total number of individuals of all species in all quadrats}} \times 100$$

$$\textbf{Relative Frequency (RF)} = \frac{\text{Number of occurrences of a species}}{\text{Number of occurrences of all species}} \times 100$$

$$\textbf{Relative Dominance (RDm)} = \frac{\text{Total basal area of a species in all the quadrats}}{\text{Total basal area of all the species in all the quadrats}} \times 100$$

Importance Value Index (IVI) : The relative values of density, frequency and dominance were summed-up to represent the Importance Value Index of all the recorded individual species i.e.

$$\textbf{IVI} = RD + RF + RDm$$

2.2.4.2. FREQUENCY DISTRIBUTION

Frequency distribution of species in the community was made following Raunkaier (1934) as :

Class A : species with frequency from 1-20 %.
Class B : " " " " 21-40 %.
Class C : " " " " 41-60 %.
Class D : " " " " 61-80 %.
Class E : " " " " 81-100 %.

Law of Frequency = A > B > C > = < D< E

This tendency shows the normal distribution of frequency percentage which was used in testing the uniformity of the vegetation.

2.2.4.3. SIMPSON'S INDEX ($\hat{\lambda}$)

Simpson's Index was calculated by using the following formula :

$$\hat{\lambda} = \sum_{i=1}^{S} \frac{ni\,(ni-1)}{n\,(n-1)}$$

where ni is the number of individuals of the ith species in all quadrats and n is the total number of individuals of all species (s) in all the quadrats.

2.2.4.4 SHANNON-WEINER INDEX ($\overline{H'}$)

Species diversity was estimated as Shannon-Weiner Index following Shannon & Weaver (1963)

$$\overline{H'} = -\sum_{i=1}^{S} \left[\left(\frac{ni}{n}\right) \text{In} \left(\frac{ni}{n}\right) \right]$$

where n*i* is the number of individuals of the *ith* species in all quadrats and n is the total number of individuals of all species (s) in all the quadrats.

2.2.4.5. HILL'S DIVERSITY NUMBERS N0, N1 AND N2

Hill's diversity indices were calculated by the following formulae (Hill, 1973)

Number 0 = **N0 = S**

where S is the total number of species

Number 1 = **N1**= $e^{H'}$

where H' is the Shannon-Weiner Index (described earlier)

Number 2 = **N2** = $1/\hat{\lambda}$

where $\hat{\lambda}$ is Simpson's Index (described earlier)

2.2.4.6. RICHNESS INDICES

Richness indices were calculated as follows:

Index 1, the Margalef (1958) index,

$$\mathbf{R1} = \frac{S - 1}{\ln (n)}$$

and **Index 2**, the Menhinick (1964) index,

$$\mathbf{R2} = \frac{S}{\sqrt{n}}$$

where S is the total number of species observed and
n is the total number of individuals observed

2.2.4.7. EVENNESS INDICES

Evenness indices were calculated using following formulae :

Evenness Index 1 (E1) : The Pielou's evenness index (Pielou, 1977),

$$\mathbf{E1} = \frac{H'}{\ln (s)}$$

where H' is the Shannon-Weiner index (mentioned above) and
S is the number of species.

Evenness Index 2 (E2) : The Sheldon's evenness index (Sheldon, 1969),

$$\mathbf{E2} = \frac{eH'}{S}$$

where H' is the Shannon-Weiner index (described earlier) and
S is the number of species.

Evenness Index 3 (E3) : The Heip's evenness index (Heip, 1974).

$$\mathbf{E3} = \frac{eH' - 1}{S - 1}$$

where H' is the Shannon-Weiner index (mentioned earlier) and S is the number of species.

Evenness Index 4 (E4) : The Hill's evenness index (Hill, 1973).

$$\mathbf{E4} = \frac{N2}{N1}$$

where N1 and N2 are the Hill's diversity numbers I and 2 (described earlier).

Evenness Index 5 (E5) : The Alatalo's modified Hill's ratio (Alatalo, 1981).

$$\mathbf{E5} = \frac{(1/\hat{\lambda}) - 1}{e^{H'} - 1}$$

where $\hat{\lambda}$ is the Simpson's Index (described earlier) and
H' is the Shannon-Weiner Index (described earlier).

2.2.4.8. SIMILARITY INDEX AND DISSIMILARITY INDEX

The Index of Similarity between two communities was calculated by using following formula (Sorensen, 1948).

$$\textbf{Similarity Index (S)} = \frac{2C}{A+B}$$

where, A = Number of species in community A,
B = Number of species in community B,
C = Number of species common to both communities.

Dissimilarity Index = 1 - S

where, S is the Similarity Index.

2.2.4.9. GENERIC COEFFICIENT

Generic coefficient of community was calculated by using following formula (Sharma, 1990).

$$\textbf{Generic coefficient} = \frac{\text{Number of genera}}{\text{Number of species}} \times 100$$

2.2.2.4.10. MATURITY INDEX

The maturity index was calculated by the formula given by Sharma (1990)

$$\textbf{Maturity Index} = \frac{\text{Total frequency}}{\text{Total number of species}}$$

2.2.4.11. INTERSPECIFIC ASSOCIATION BETWEEN *Streptocaulon sylvestre* WIGHT AND OTHER ASSOCIATE SPECIES.

Interspecific association was analysed between *Streptocaulon sylvestre* and other associate species based on the presence and absence data of each species in sampled quadrats at Site- I and II. The data obtained were arranged in 2×2 contingency tables for each pair of species and then the type of association, Chi-square test statistics and indices of association were computed.

Type of Association : Cole (1949) proposed the positive and negative association between pair of species as

Positive association = When, *ad* greater than *bc*

Negative association = When, *ad* less than *bc*.

where, *a* is the number of quadrats where both species occur.

b is the number of quadrats where species A (S. sylvestre) occurs, but not B (associate)

c is the number of quadrats where species B occurs, but not A.

d is the number of quadrats where neither A nor B are found.

when *ad* is equal to *bc*, it is estimated as no interaction.

Chi-square (χ_1^2) = The Chi-square test statistic was computed by the formula given by Ludwig & Reynolds (1988) as :

$$\chi_1^2 = \frac{N\,(ad - bc)^2}{mnrs}$$

where N is the total number of quadrats ($N = a + b + c + d$), *a, b, c* and *d* as described above, *m* is the $a + b$, *n* is the $c + d$, *r* is the $a + c$ and *s* is the $b + d$.

The significance of the Chi-square test statistic was determined by comparing it to the theoretical Chi-square distribution.

Indices of Association : Three association indices were measured by using following formulae.

Ochiai Index (OI) : Ochiai (1957) proposed the index as

$$OI = \frac{a}{\sqrt{a+b}\sqrt{a+c}}$$

Dice Index (DI) : Dice Index was calculated as per Dice (1945) using the following formula :

$$DI = \frac{2a}{2a + b + c}$$

Jaccard Index (JI) : Jaccard index was calculated by using following formula (Jaccard, 1908)

$$JI = \frac{a}{a + b + c}$$

a, b, c and *d* as defined above.

2.3. PHENOLOGY AND BIOLOGICAL SPECTRUM

Repeated visit to different localities in different months were made to record the stages of life cycle of different species of plants in the vegetation and a comprehensive phenological calender was prepared, covering the period from July 1997 to May 2001. Six phenological stage were recorded viz. seedling appearance phase, vegetative growth phase, flowering phase, fruiting phase, seed dispersal phase and death of the annuals and dormancy of perennials. A species was considered in a particular phenophase when it actually entered in that stage for instance, when just one or a few individuals starts flowering, plants enter in the phase / stage of flowering. Detailed observations were made regularly in natural conditions.

Phenological observations on *Streptocaulon sylvestre* were carried out, both in the experimental garden of Botany Department, North Bengal University as well as in its natural habitat. The phenological observations viz. germination, sprouting, different stages of vegetative growth of seedling flowering, fruiting, seed dispersal and death of areal parts were recorded.

For life-form classification, Raunkiaer 1934, has been followed, without any modification, as follows :

Phanerophytes : Perennating buds well above the ground.

Chamaephytes : Herbaceous perennials or suffrutescent plants bearing perennating buds just above the ground level to 30 cm high.

Hemicryptopytes : Perennating buds half hidden at the ground level.

Cryptophytes : Perennating organs below the ground surface

Therophytes : Annuals which propagate through seeds or spores and complete their life cycle within a short period.

2.4. EDAPHIC FEATURE

Soil samples from 0-10, 10-20 and 20-30 cm depths were collected from each of the four sites in polyethene bags and transported to the laboratory. Soil colour, texture, pH, electrical conductivity and soil moisture content were then determined immediately.

To get a good representative sample, repeated quataring technique was employed. Moreover, ***Streptocaulon sylvestre*** is now growing in the habitat of Site-I and II only (as mentioned in detail in Chapter-I). The number of soil samples were, therefore, collected from immediately around the roots of this species. Collected samples were then dried under shade, crushed and carefully strained first through

5 mm sieve and then through 2 mm sieve. The materials retained in the 2 mm sieve were used as samples. An attempt was made to remove root lets and rocky materials completly before final storage. The samples were preserved carefully in p.v.c. container. These samples were then analysed for soil mechanical composition, organic matter, total nitrogen, available phosphorus, potassium, iron, manganese, copper and zinc.

Soil colour was compared with the Munsell colour chart and the values were recorded for each samples under dry as well as wet conditions. Soil texture was determined through a rapid procedure by feel and rubbing. Mechanical composition i.e. sand, silt and clay percentage in soil was determined by the Hydrometer method proposed by Bouyoucos (1927).

Soil pH was measured by a digital pH meter (1:2, soil : water). Electrical conductivity was measured using an A.C. salt bridge or electrical resistance bridge and conductivity cell having electrodes coated with platinum black.

Percentage of soil moisture was determined by using the following formula :

$$\textbf{Percentage of soil moisture} = \frac{W_1 - W_2}{W_2} \times 100$$

where W_1 = Weight of fresh (wet) soil

W_2 = Weight of oven dry soil.

Determination of soil moisture content was made at the interval of one month throughout the year.

Field capacity was determined by saturating the soil sample and allowing the water to drain down for 2 days. The atmosphere above the soil surface however, was kept humid to avoid loss of water due to evaporation. The moisture content as a percentage of oven dry weight of soil was then determined to get the field capacity value.

For organic matter Walkey & Black's (1934) rapid titration method was used. A general approximation of total nitrogen content was made from organic matter content of soil following Jackson (1958) as follows :

Nitrogen (N) = Organic matter × 0.05

This approximate factor is subject to great change with widely different soils.

Available phosphorus was determined by using the method of Bray & Kurtz (1945). Available potassium was estimated by extraction method in I N NH_4O AC as described by Jackson (1958).

Four microelements viz. iron, manganese, copper and zinc were estimated in a single extraction with D T P A (Diethylene-Triamine Penta Acetic Acid) by using Atomic Absorption Spectrophotometer.

2.5. SEED GERMINATION AND SEEDLING SURVIVABILITY OF *Streptocaulon sylvestre* WIGHT.

In this part of work, fifteen types of investigations were carried out on seed characters, seed germination, seedling growth, reproductive capacity and seedling survivality of *Streptocaulon sylvestre* Wight. Mature fruits of *S. sylvestre* were collected from the campus of the University of North Bengal, where it is growing now, in the months of November and January during 1997 to 2000. After harvesting the seeds were separated, air dried and stored in desicator with Silica gel under normal diurnal temperature in brown paper bags. Periodically they were taken out and tested for germination at room temperature. The seeds were separated from the hairs of coma (as germination was not affected by the presence on absence of coma) manually and were surface sterilised with 1.0 % mercuric chloride solution and washed for 1 h with distilled water. Radicle emergence was considered as a criterion for seed germination and the total number of germinated seeds were recorded daily. Following investigations were conducted.

2.5.1. FRUIT AND SEED CHARACTERS

For determination of fruit and seed characters fruits from 30 plants were collected separately at random and counted separately. Shape, size, length and circumference of each fruit were recorded. All the seeds from each fruit of each plant were collected, counted and air dried. The morphology of seed was studied by recording the seed coat colour, the differences in seed shape & size, the seed length & breadth and the length of hairs of coma. Weight of 100 dry seeds and coma was determined according to the size of the seed. An Electronic Digital Weigh Machine (SARTORIUS Model no - BP 110) was used for this purpose.

2.5.2. SEED VIABILITY

Seed viability was followed immediately after harvest i.e. without storage the seeds were kept for germination and after every four months the experiment was repeated for fortyfour months of dry storage. 25 seeds were placed in a sterile glass petridish containing a single layer of filter paper (Whatman no. -1) soaked with distilled water. Four replicates were taken each time for viability testing.

2.5.3. EFFECT OF SEED SIZE ON GERMINATION

Seeds were graded into large, medium and small size and germinated on single layer moist filter paper soaked with distilled water in petridishes separately. Four sets of 25 seeds each were taken for each treatment.

2.5.4. SEED GERMINATION UNDER DIFFERENT TEMPERATURES

Seeds were placed in glass petridishes on single layer of moistened filter paper and kept at 0, 10, 20, 25, 30, 35 and 40° C temperature with 8 hrs. exposure to light (per day). Each treatment had four replicates of 25 seeds each. Experiments were conducted in refrigerator and in incubator.

2.5.5. EFFECT OF LIGHT PERIOD ON GERMINATION

The experiments were performed in petridishes containing moist filter paper (Whatmer no. 1) and were exposed to various light period as given below -

(i) continuous dark
(ii) 8 hrs light per day,
(iii) 12 hrs light per day,
(iv) 16 hrs light per day,
(v) continuous light (24 hrs.) and
(vi) diffuse or day light.

The artificial light was provided from one 100 watt incandescent bulb and two fluorescent tubes of 20 watt in a dark room. Green bulb light was used as safe light and dark conditions was created by wrapping petridishes with two layers of black carbon papers. Each treatment had four replicates of 25 seeds each and distilled water was used for moistening the substrata as and when it was essential.

2.5.6. SEED GERMINATION AT DIFFERENT POSITION AND ORIENTATION IN SOIL AND SAND

Soil and sand were used as substratum for germination behaviour in these two experiments. Field soil and river sand were taken separately in wide mouth earthern pots and seeds were planted in vertical, horizontal and inverted orientation onto the surface and at 2 cm depths of the uniform layer of moist soil and sand. Some amount of water was added daily to maintain appropriate level of moisture condition. Each treatment was replicated four times with 25 seeds each. Seedling emergence was observed and recorded daily and the germination percentage was expressed on the basis of emerged seedlings only.

2.5.7. SEED GERMINATION IN WATER LOGGED SOIL AND SAND

River sand and soil from the habitat of *S. sylvestre* were taken separately in plastic tray and seeds were sown into the surface and 2 cm depth in water logged

condition. Soil and sand were watered daily maintaining appropriate waterlogging condition, depending on the need. Each treatment had four replicas of 25 seeds each. Seedling emergence was observed daily.

2.5.8. SEED GERMINATION AT DIFFERENT pH LEVELS

The solutions of six different pH values viz. 4, 5, 6, 7, 8 and 9 were prepared by adjusting the pH of distilled water (pH 7) with either a solution of 0.1 N HCl (for pH 4, 5 and 6) or a solution of 0.1 N NaOH (for pH 8 and 9). To plant seeds, one Whatman No-1 filter paper was placed in a glass petridish. Seven ml of solution were added to each petridish at the beginning of the experiment and some amount was added in subsequent days to maintain the appropriate moisture level. Four petridishes for each pH value were prepared and 25 seeds were incubated in each dish.

2.5.9. EFFECT OF SODIUM CHLORIDE ON SEED GERMINATION AND SEEDLING GROWTH

Seven concentrations of Sodium Chloride solution viz. 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.00 g/l were chosen and prepared by dissolving Sodium Chloride (NaCl) in distilled water. For each treatment five replicates of 10 seeds each were used. Seeds were placed in petridishes with single layer of filter paper (Whatman No-1) and moistened with equal volume (approximately 10 ml) of respective NaCl solution. A controlled set with plain distilled water was kept along with it. Daily counts of seed germination were made and root & shoot length of seedlings were recorded after 12 days of sowing.

2.5.10. EFFECT OF GROWTH REGULATORS (VIZ. GA_3, BAP, IAA & 2,4 - D) ON SEED GERMINATION AND SEEDLING GROWTH

The seeds were tested for germination and seedling growth behaviour in the different concentrations of GA_3 (Gibberellic Acid), BAP (6-Benzyl amino Purine), IAA (Indole acetic Acid) and 2,4-D (2, 4 - Dichlorophenoxy acetic acid). Each growth regulator had six different concentrations viz. 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg/l and each treatment had five replicates of 10 seeds each. The experiments were performed by petridish method lined with single layer of filter paper and moistened with equal volume of test solution (approximately 10 ml) along with distilled water control. Observation on seed germination and seedling growth behaviour were recorded daily and root & shoot length were measured after 12 days of sowing.

2.5.11. REPRODUCTIVE CAPACITY AND SEEDLING SURVIVABILITY

Reproductive capacity was determined by calculating the average seed output per plant and average percentage of seed germination. For determination of seed output, fruits produced by 30 plants were collected at random and counted separately. All the seeds from each fruit of each plant were collected and counted and after air drying stored properly. In addition, number of total plants, number of flower producing plants, and number of fruits producing plants were observed and counted at every 15 days for one year in marked areas.

To determine the percentage of germination and percentage of survival of seedlings, four nursery beds of 1.0 m × 1.0 m were prepared. Seeds were sown at the rate of 50 per plot during June and watered to maintaining appropriate moist condition, depending on the need. Observation on seedling emergence was recorded daily for 20 days. Number of saplings present, 10 weeks after sowing were counted and the percentage survival of seedlings was calculated.

2.5.12. DATA ANALYSIS

The results of different investigations were statistically analysed at the replication and treatment levels as mean and standard error (±). Using the data from different investigations following parameters were calculated:

2.5.12.1. SEED SIZE INDEX AND SHAPE INDEX

Seed size index and shape index were calculated following the methods of Hill *et al.*(1986)

Seed size Index = Length of seed × breadth of seed

$$\text{Seed shape Index} = \frac{\text{Length of seed}}{\text{Breadth of seed}}$$

2.5.12.2. GERMINATION PERCENTAGE : Germination percentage was calculated by using the following formula.

$$\text{Germination \%} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

2.5.12.3. GERMINATION PERCENTAGE INHIBITION OR STIMULATION

Germination percentage Inhibition or Stimulation was calculated as per the following formula.

$$\textbf{Germination \% Inhibition or Stimulation} = \frac{\text{Germination \% in desired solution - Germination \% in control}}{\text{Germination \% in control}} \times 100$$

2.5.12.4. NONVIABLE PERCENTAGE

Percentage of non viable seeds was calculated by using the following formula.

$$\textbf{Nonviable \%} = \frac{\text{Total number of non viable seeds}}{\text{Total number of seeds sown}} \times 100$$

2.5.12.5. INDEX OF SPEED OF GERMINATION

The following formula as given by Agrawal (1995) was used for calculating the index of speed of germination.

$$\textbf{Index of Speed of Germination} = \sum \frac{\text{Quotients of daily counts}}{\text{Number of days of germination}}$$

2.5.12.6. PERCENTAGE OF INHIBITION OR STIMULATION OF SHOOT LENGTH

Percentage of Inhibition or Stimulation of shoot length was calculated by using the following formula.

$$\textbf{\% Inhibition or Stimulation of Shoot length} = \frac{\text{Shoot length in desired solution - Shoot length in control}}{\text{Shoot length in control}} \times 100$$

2.5.12.7. PERCENTAGE OF INHIBITION OR STIMULATION OF ROOT LENGTH

The following formula was employed to find out the percentage of Inhibition or Stimulation of root length.

$$\textbf{\% Inhibition or Stimulation of Root length} = \frac{\text{Root length in desired solution - Root length in control}}{\text{Root length in control}} \times 100$$

2.5.12.8. PERCENTAGE OF INHIBITION OR STIMULATION OF SEEDLING LENGTH

Percentage of Inhibition or Stimulation of Seedling length was calculated by using the following formula.

$$\text{\% Inhibition or Stimulation of Seedling length} = \frac{\text{Seedling length in desired solution - Seedling length in control}}{\text{Seedling length in control}} \times 100$$

2.5.12.9. SHOOT VIGOUR INDEX

The Shoot Vigour Index was calculated as per the following formula described by Thind & Malick (1988).

Shoot Vigour Index = Germination % × Shoot length

2.5.12.10. ROOT VIGOUR INDEX

Root Vigour Index was calculated by using the following formula

Root Vigour Index = Germination % × Root length

2.5.12.10.11. SEEDLING VIGOUR INDEX

Seedling Vigour Index was calculated by using the following formula

Seedling Vigour Index = Germination % × Seedling length

2.5.12.12. SHOOT : ROOT RATIO

The Shoot : Root ratio was calculated by using the following formula (Bajpai *et al.*, 1995).

$$\textbf{Shoot : Root Ratio} = \frac{\text{Shoot Length}}{\text{Root Length}}$$

2.5.12.13. REPRODUCTIVE CAPACITY AND SEED OUTPUT

The Reproductive Capacity and Seed Output were determined by standard method (Salisbury, 1942).

$$\text{(a) Reproductive Capacity} = \frac{\text{Average seed output of a plant} \times \text{\% of germinated seeds}}{100}$$

(b) Average Seed Output =Average number of fruits per plant × Average number of seeds per fruit

2.5.12.14. PERCENTAGE OF SURVIVABILITY OF SEEDLINGS

Percentage of Survivability of Seedlings was calculated by using the following formula.

$$\textbf{\% Survivability of Seedlings} = \frac{\text{Number of saplings present}}{\text{Number of seedlings produced}} \times 100$$

2.5.12.15. PERCENTAGE OF MORTALITY OF SEEDLINGS

Percentage of Mortality of Seedlings was calculated by using the following formula.

$$\textbf{\% Mortality of Seedlings} = \frac{\text{Number of deaths of seedlings}}{\text{Number of seedlings produced}} \times 100$$

2.5.12.16. AGGRESSIVE CAPACITY

The following formula as given by Reddy & Aruna (1997) was used for calculating the Aggressive Capacity.

$$\textbf{Aggressive Capacity} = \frac{\text{Reproductive Capacity} \times \text{\% Survivability of seedlings}}{100}$$

2.6. *IN VITRO* REGENERATION OF *Streptocaulon sylvestre* WIGHT

Mature seeds of *Streptocaulon sylvestre* Wight collected from North Bengal University campus during January 1999 were used as explant material for *in vitro* seed germination, while the *in vitro* germinated seedlings were used as explant source for callus induction. Healthy and uniform seeds were first washed with Tepol (BDH) under running tap water and then washed again at least thrice with double distilled water. The washed seeds were surface sterilized by agitating them thoroughly in 70 % ethanol for 1-2 min followed by rinsing in double distilled water and then treated with 2-3% sodium hypochloride (NaOCl) for 15 min and washed 5-6 times with sterilized double distilled water. These seeds were germinated aseptically on MS basal medium (Murashige & Skoog, 1962). *In vitro* germinated seedlings were collected aseptically from the conical flasks and used directly without sterilization.

2.6.1. CULTURE MEDIA

The culture medium composition formulated by Murashige & Skoog (1962) as described in the Table-2.2 was used for seed germination and callus induction.

2.6.2. PREPARATION OF CULTURE MEDIUM

A convenient approach to medium preparation for most operations is to have a series of stock solutions. The stock solution concentrations required for MS medium were made as indicated in the Table-2.2. The macro element stock solutions were prepared at 10 × of the final concentrations and for micronutrients the stock solutions was prepared at 100 × of final concentration. All the stock solutions were kept in a refregerator.

For making 1000 ml of culture medium, 100 ml solution from each of macro elements stock solutions and 10 ml from each micro element stock solutions were taken. The volume was then made upto 900 ml by adding double distilled water. 20 gms of sucrose (BDH) was then added and mixed properly by hand shaking and the volume was made up to 1000 ml by adding double distilled water. When the sucrose was fully dissolved the pH of the culture medium was adjusted to 3.6, 4.00, 5.4, 5.60, 5.80, 6.20 and 7.00, 0.8 - 1 % of agar (Difco) was then added slowly to the gently boiling and stirring solution. Medium having different pH (as mentioned above) was then distributed (15 and 25 ml in culture tube and conical flask, respectively) in culture tubes and conical flasks. The cotton plugged culture tubes and flasks were autoclaved at 110 kPa for 20 min at 120 °C. The conical flasks with sterilized medium were cooled and used for *in vitro* seed germination.

To make each 1000 ml of culture solution for the induction of callus, 100 ml solution from each of the six macro element stock solutions and 10 ml from each of the micro element stock solutions were taken and the volume was made upto 900 ml by adding sterilized double distilled water. The pH of the solution was adjusted to 5.4 - 5.8. 20 gms of sucrose (BDH) was then added and by adding sterilized double distilled water the volume was made 1000 ml. 8 to 10 gms of agar (Difco) was then added slowly to the gently boiling and stirring solution. When the agar was fully dissolved 20 ml and 50 ml of medium was poured in each culture tubes and 100 ml conical flasks (Borosil) respectively. The tubes and conical flasks were plugged with cotton and then autoclaved at 110 kPa for 20 min at 120 °C. Different concentrations of growth regulators i.e. IAA, 2,4-D and NAA were incorporated into nutrient media under aseptic condition in a laminar air flow cabinet. The conical flasks and tubes with sterilized medium were then cooled as slants and used for callus induction.

Table-2.2 Murashige & Skoog basal medium (1962)

Name of chemicals	Amount required per Lt of culture med.	Stock soln. conc.	Amount of stock soln. to be taken
MACRO ELEMENTS (× 10)			
Ammonium Nitrate	1650 mg	16.5 gm	100 ml
Potassium Nitrate	1900 mg	19 gm	100 ml
Magnessium Sulphate	370 mg	3.7 gm	100 ml
Calucium Chloride	440 mg	4.4 gm	100 ml
Potassium Phosphate	170 mg	1.7 mg	100 ml
MICRO ELEMENTS (× 100)			
Manganese Sulphate	22.3 mg	2.23 gm	10 ml
Zinc Sulphate	8.6 mg	0.86 gm	10 ml
Cupric Sulphate	0.025 mg	0.0025 gm	10 ml
Potassium Iodite	0.83 mg	0.083 gm	10 ml
Cobalt Chloride	0.025 mg	0.0025 gm	10 ml
Boric Acid	6.2 mg	0.62 gm	10 ml
Sodium Molybdate	0.025 mg	0.0025 gm	10 ml
Ferrous Sulphate	27.85 mg	2.785 gm	10 ml
EDTA	37.25 mg	3.725 gm	10 ml

2.6.3. GROWTH REGULATORS

Hormones used were prepared fresh. For preparing 10 ml of auxin (IAA, 2,4-D and NAA) solutions, 5 mg of each of the Auxins (Sigma) was dissolved in 1 ml of 90 % ethanol in a clean autoclaved test tube (Borosil) and the volume was made upto 10 ml by adding sterilized double distilled water and kept in a refrigerator. Subsequently auxin solutions were diluted as per the requirement of the experimented volume (i.e. 0.2, 0.5, 1.0, 2.0 and 3.0 mgl^{-1} with sterilized double distilled water before adding in the medium.

2.6.4. INOCULATION AND CULTURE CONDITION

Surface sterilized seeds (5 in each flask) were inoculated aseptically and carefully into conicl flasks containing medium. The flasks were kept in dark till germination and maintained at a regulated temperature of 25 ± 2 °C (as germination was accelarated in dark condition *in vivo* experiment). After germination they were placed under 16 hr

photoperiod from cool white lights giving 1000 Lux at culture level. The humidity was maintained at 70 ° ± 5 % relative humidity. Observations were made at regular intervals. Emergence of radicle was considered as the first visible symptom for germination.

In vitro grown 20-30 days old seedlings were taken out aseptically from the conical flasks where they were growing. Cotyledon, hypocotyle and epicotyle were separated with the help of a sharp sterilized blade and inoculated aseptically in the culture tubes and conical flasks containing culture medium.

The whole inoculation process was performed under aseptic condition in a laminar air flow cabinet. The scalpels, forceps and other appliances used in this process were autoclaved at 110 K Pa for 20 min at 120 °C and also sterilized by dipping in rectified spirit and flaming prior to every use. All cultures were incubated at 25 ° ± 2C under 16 hr photoperiod (in a 24 hr cycle) from cool white fluorescent tubes giving 1000 lux at culture level. The humidity was maintained at 70 ± 5 % relative humidity.

The observations on the effect of different medium were made on visual basis at regular intervals. Five explant relicates on each attempt were used to initiate the experiment.

Subsequent experiments were conducted to find out suitable pH of the media for optimum seed germination. For optimal callus growth, concentrations of hormons and suitable explants were also evaluated.

Germination percentage was calculated by using the following formula :

$$\frac{\text{Seeds germinated}}{\text{Total no. of seeds / flask}} \times 100$$

2.7. ALLELOPATHIC EFFECT OF ASSOCIATES ON *Streptocaulon sylvestre*

The seeds of *Streptocaulan sylvestre* Wight were collected from North Bengal University campus in the months of December and January during 1997 to 2000. Leachates individually or in mixture and extract of associate plants species were used in seed bioassay to determine the allelopathic effect . The associate species viz. *Borreria alata, Carex indica, Cymbopogon pendulus, Desmodium triflorm, Elephantopus scaber, Lindernia crustacea, Mitracarpus verticillatus, Phyllanthus virgatus, Phyllanthus urinaria, Pueraria phaseoloides, Mnesithea laevis, Rungia pectinata, Saccharum spontaneum, Sporobolus indicus and Vernonia cinerea* were collected from the habitat of *Streptocaulon sylvestre* during 1998 to 2000 and washed thoroughly in distilled water for three times. For this work, procedures described by Datta & Ghosh (1987) was followed . Leachates of areal parts or whole plant were made by directly soaking 100g of fresh material of each species in distilled water for 72 hours. Each type of leachates were filtered through

Whatman (No.1) filter paper and made upto 250ml by adding more distilled water. This became stock solution of leachates and was expressed by the ratio 1:2.5. Extract stock solutions, 1:2.5, were also prepared following the same procedure, only extraction was done in a Bajaj Mixture Machine. From the constituted stock solution (1:2.5) of leachates or extracts desired concentrations i.e. 1:5, 1:10 and 1:20 were prepared by the way of subsequent dilution with distilled water. Mixed leachates solutions were prepared by the combination of equal volume of same concentrations of different leachates. The pH of the test solution was measured by using digital pH meter.

Seeds of *S. sylvestre* were surface sterilised with 0.1% Mercuric Chloride solution for five minutes and washed thrice with distilled water. 25 healthy seeds were placed in a sterile 15cm glass petridish lined with single layer of filter paper. The filter paper was moistened sufficiently by adding equal volume (approximately 10 ml) of respective test solutions. For each treatment, there were four replicates and one set control with sterile distilled water. The petridishes were arranged in randomised design and were kept under 25 ° ± 2 °C temperature. The germination of seeds were counted daily and the linear length of roots, shoots and seedlings were made after 12 days of sowing. Emergence of redicle was considered as a criterion for seed germinaion. Lateral roots initiation were also counted and recorded.

2.7.1 DATA ANALYSIS

The data of different experiments of allelopathic effect on seed germination and seeding growth were statistically analysed at the replication and treatment levels as mean and standard error (±) and presented in tabular forms.

Seed Germination Percentage, Germination Percentage Inhibition or Stimulation, Nonviable Percentage, Index of Speed of Germination, Percentage of Inhibition or Stimulation of Shoot Length, Percentage of Inhibition or Stimulation of Root Length, Percentage of Inhibition or Stimulation of Seedling Length, Shoot Vigour Index, Root Vigour Index, Seedling Vigour Index and Shoot : Root Ratio were calculated following the same formulae as described earlier in 2.5.12.

Percentile of viability was calculated by using the following formula

$$\textbf{Percentile of Viability} = \frac{\text{Percentage of viability of observed dilution}}{\text{Maximum percentage of viability}} \times 100$$