Rauwolfia serpentina (L.) Benth. Ex Kurz. is commonly known as Sarpagandha (Indian snakeroot). It is an important medicinal plant found in Indian subcontinent and south East Asian countries. Generally, it grows in the region with annual rainfall of 200 to 250cm at 1000 m altitude. The deep fertile soil with rich organic matter is favorable for growth of this plant. The major causes of declination of this plant species from natural habitat are poor rate of seed germination, over exploitation and habitat loss. (Dey and De, 2010).

1.1: History:

Rauwolfia serpentina historical story is a most fascinating one. It is mentioned in ancient literature in Charaka (1000-800 BC). In the Sanskrit it is called as Sarpagandha.

It is used by Hindus from centuries as a febrifuge and as an antidote against the bites of poisonous snakes and insect stings. It also used to treat dysentery, painful infections of the intestinal canal, uterine contraction and to promote expulsion of fetus. It was also mentioned as a stomachic cures fever. The root was used as a remedy for painful affections of the bowels in the Western parts of India. Because of the drug’s noted sedative effects, it was used to treat over a million Indians for high blood pressure. Its generic name was coined in the seventeenth century by French botanist, Plumeres in honour of the well known sixteenth century German physician, botanist, traveler and author Leonard Rauwolf of Ausburg. In the last decade its medicinal value has been accepted by the allopathic system. In 1952, an U.S. physician named Wilkins demonstrated the positive effects of reserpine, one of 50 isolated from the root, was to revolutionize the treatment of mental illness and high blood pressure by the western medical. The snakerooot plant has been
used as a part of Ayurvedic medicine since thousands of years. (Bein and Mukerji, 1965, Shah et al., 1965, Pandey, 1978 and Himalayhealthcare.com).

1.2: Origin and Distribution:

*Rauwolfia serpentina* is found indigenous to the moist, deciduous forests of South East Asia including Burma, Bangladesh, Bhutan, China, India, Myanmar, Nepal, Pakistan, Thailand, Viet Nam, Sri Lanka, Malaysia, Andaman Islands and Indonesia. Most of the drug is obtained from wild sources in these countries. Dey and De, (2010) and Kavita, (2005).

It is cultivated on a small scale in India and Bangladesh. In India, it is found in the central region, i.e. between Sirmor and the Gorakhpur district of Uttar Pradesh, in shady, moist or sometimes swampy localities. In the east Bihar, North Bengal and Assam as well as in Khasi, Jaintia and Gharo Hills and plant is encountered more commonly on the forest margin of mixed deciduous forests. In the Western Ghats, it occurs more frequently in Goa, Coorg, the North Kanara and Shimoga districts of Karnataka and Palghat, Calicut and Trichur in Kerala. In Orissa, Andhra Pradesh and Himachal Pradesh the areas comprising the catchments of the river Godavari are the richest. The plant is chiefly associated with Sal (*Shorea robusta*) forests as well as bamboo brakes. A major part of the commercial supply of this drug used in the USA and European countries originates from India, Pakistan, Sri Lanka, Myanmar (Burma) and Thailand, with India being the major supplier. The present day commercial supplies of the roots of Sarpagandha are mostly from Uttar Pradesh, Bihar, Orissa, West Bengal, Assam, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and Maharashtra. *Rauwolfia serpentina* roots exhibit more or less the same morphological features but they differ in
their alkaloid content, not only in geographically distinct areas but also within the same geographical area. (Pandey, 1978 and www.gardenguides.com/herbs/rauwolfia).

There is also a great demand for the alkaloids as well as the raw drug in the international market. The world requirement of dried Sarpagandha roots is around 20,000 ton/year. The consumption of the raw drug is substantial in the indigenous drug market. Steps, therefore, will have to be taken to increase the present production to about 100-150 t/annum of dry roots. This is possible only if the plant is brought under large-scale cultivation in agro climatically suitable areas. (Kavita, 2005).

1.3: Morphology of the Plant:

The Sarpagandha plant is an evergreen perennial under shrub, growing to a height of 60-90 cm. Its leaves are simple, 7.5 cm long and 3.5 – 5 cm broad, elliptic or lanceolate, glabrous, bright-green above and pale green beneath pointed and occurring in whorls of 3-5. The inflorescence is a many flowered corymbs with white or pink flowers. The fruit is a drupe, 0.5 cm in diameter and shiny black when fully ripe. The root system consists of a prominent, tuberous, soft tap-root. The root system consists of a prominent, tuberous, soft tap-root. The root system consists of a prominent, tuberous, soft tap-root reaching a length of 30-50 cm in a 2 year old plant. Its diameter at the thickest portion varies from 1.2 to 2.5 cm. The root-bark, which constitutes 40-60% of the whole root, is rich in alkaloids. The fresh roots emit a characteristic acrid aroma and are very bitter in taste. (Dey and De, 2010., Bein and Mukerji, 1965).
1.3.1: **Classification of Rauwolfia serpentina**: Borsa, (2010).

Kingdom: Plantae
Family: Apocynaceae
Genus: Rauwolfia
Species: *serpentina*

Scientific Name: *Rauwolfia serpentina*

1.3.2: **Common name in different languages**: (Dey and De, 2010 and Pandey, 1978).

- Hindi - Chandrabhaga, Chota-chand, Sarpagandha
- English - Rauvolfia / Indian snakeroot
- Latin - *Rauwolfia serpentina*
- Sanskrit - Sarpaghandha
- Tamil - Chevanamalpodi, Sarpagandha
- Kannada - Keramaddinagaddi
- Telgu - Patalaguni, Patalagandha, Sarpagandha
- Malayalam - Churannavilpori, Suvapavalporiyam., Amalpori
- Marathi - Harkaya, Harki, Hadaki/Adakai
- Assamese: Arachoritita
- Bengali: - Chandra
- Kannada: - Sutranabhi
- Chinese: - Lu fu mu
- Arunachal Pradesh: - Bhungmaraja
- Ceylon: - Acaverya
- Java: - AkarTikoes, Poel Pandak
- Gujarati: - Amelpodee
Fig.1: *Rauwolfia serpentina* plants.
1.4: Species of Rauwolfia:

There are approximately 85 species of the genus *Rauwolfia* mainly found in tropical regions. *Rauwolfia serpentina*, *R. caffra*, *R. tetraphylla*, *R. micrantha* and *R. vomitoria* are some of the important species of Rauwolfia. The Reserpine content in the root of *Rauwolfia serpentina* has attracted worldwide attention for drug development. *Rauwolfia serpentina* contains more than 50 alkaloids including the therapeutically important reserpine, rescinnamine, deserpidine, ajmalacine, ajmaline, neoajmalin, serpentine, and yohimbine. The most important species of Rauwolfia are described as follows. (Hines, Eckman, 1993).

1.4.1: *Rauwolfia serpentina* (L.) Benth. ex. Kurz.

*Rauwolfia serpentina* (L.) Benth. ex. Kurz. is an under shrub, belongs to *Apocynaceae* family of dicotyledonous. It is indigenous to India and other tropical countries of Asia and is pan tropic in distribution. This species is declared as endangered. It is known as sensitive species of Rauwolfia.

1.4.2: *Rauwolfia caffra*:

*Rauwolfia caffra* is a tree which grows up to 35 m in height and its diameter is 1.5 m with a dense crown, bole straight, slightly buttressed. The bark light brown or grayish-white with irregular fissures; slash cream, exuding bitter white latex. The leaves in crowded whorls, simple, stipules absent, blade oblanceolate to linear oblanceolate, 6-32 x 1.5-7 cm, apex obtuse to acute or sub acuminate, base cuneate, margins entire, glabrous, shiny green above, paler below and lateral nerves 18-30 pairs. The petiole is 0.5-6 cm long. Inflorescences are terminal compound umbel, peduncle is 2 to 6 cm long, bracts minute; flowers bisexual, 5-merous, white, pedicles 1 mm long. Calyx cup shaped, 1 mm long, 5-toothed or lobed; corolla salver-shaped, white, tube 3-4.5 mm long, lobes
ovate, 1 mm long, mouth filled with whitish hairs. The stamens 5, inserted above the middle, ovary of 2 more-or-less united carpels, often only 1 developing. Fruits are subglobose to ovoid drupe, smooth and green at first, becoming wrinkled. The two seeded fruits are blackish purple, 1-1.5 cm long, 2 cm in diameter and seeds 1 or 2, white, ovoid-compressed, endosperm fleshy. It is naturally distributed from Tanzania to the Eastern Cape of South Africa, occurring near rivers and streams. It is known as indicator of water when growing away from rivers and streams it is always associated with available ground water. (Hines, Eckman, 1993 and Beentji, 1994).

1.4.3: *Rauvolfia tetraphylla*:

It is very common species known as Devil Pepper, Be Still Tree, American serpent wood, devil root and milk bush. It is native of Tropical America. It is a small tree or shrub. It grows up to height of 6 ft. The leaves are acute in shape, medium to dark green in color, and occur in groups of 4 unequally-sized leaves at each node. The flowers are white coloured and very small up to length of 5 mm. leaves develop in the late summer to early fall. The leaves are used in urinary retention, epilepsy, insomnia, wounds, and fever. (www.wikipedia.com)

1.5: Phytochemical constituents:

Serpentina has number of therapeutic properties so it is an important medicinal plant in the pharmaceutical world. It is used for curing various disease and disorders due to the presence of alkaloids, carbohydrates, flavonoids, glycosides, phlobatannins, phenols, resins, saponins sterols, tannins and terpenes. The different parts of *Rauwolfia serpentina* i.e., roots, leaves and rhizomes have been used since centuries in Ayurvedic medicines for curing many diseases such as high blood pressure, mental agitation, epilepsy, traumas, anxiety, excitement,
schizophrenia, sedative insomnia and insanity. It contains more than 50 different alkaloids which belong to the monoterpenoid indole alkaloid family. It shows the presence of Ajmaline, ajmalicine, ajmalimine, deserpidine, indobine, indobinine, reserpine, reserpiline, rescinnamine, rescinnamidine, serpentine, serpentinine and yohimbine i.e., major alkaloids. *R. serpentina* also having antimicrobial, antifungal, anti-inflammatory, antiproliferative, antidiuretic and anticholinergic properties. In addition to allopathy, herbal medicine is still the basis of primary health care for 75–80% of the world population due to its certain important properties like cultural acceptability, better compatibility with the human body and lesser side effects. These facts clearly indicate that still there is a need for us to search alternative, naturally available remedies for curing millions of people worldwide. By considering these characteristic features present review aims to evaluate the various pharmacological, phytochemical and therapeutic properties of *R. serpentina* has been carried out by (Reeta Kumari, et al., 2013). Among these alkaloids, reserpine is therapeutically very important.

1.6: Reserpine:

Muller Schiltter and Bein has been isolated reserpine first in 1952 and its formula is \( C_{33} H_{40} N_{2} O_{9} \). (Gawade and Fegade, 2012).

1.6.1: Structure of Reserpine

There are different alkaloids are produced due to enzyme strictosidine synthase in *Rauwolfia serpentina* mainly the indole alkaloids. Among these, reserpine is most important of which Tryptamine and the monoterpenoid biosynthesis as shown in fig.2
1.6.2: Pharmacological effects of alkaloids of Rauwolfia:

The different pharmacologic attributes of *Rauwolfia serpentina* are as follows. (Siddiqui and Siddiqui, 1931).

1. By action on the vasomotor centre, it leads to generalized vasodilatation, with a lowering of blood pressure.
2. By depressant action on the cerebral canters, it soothes the general nervous system.
3. It stimulates the bronchial musculature.
4. Reserpine group causes stimulation of the heart and Ajmaline has been reported to stimulate respiration and intestinal movements.
5. Isoajmaline and neoajmaline causes lowering of blood pressure in intact, spinal and vertebrate animals.
6. Muller and associates (1952) and found on the basis of animal experiments, that a new alkaloid Reserpine possess marked and long lasting hypotensive activity.

7. Goto (1954), found the alkaloid Reserpine effective in 12 out of 15 cases of hypertension.

8. Vakil (1953) reported a good hypotensive response to the alkaloid Reserpine in 72% of cases, and few side effects.

9. Guy Lemieux, Andre Davignon and Jacques (1956) reported that orally administered Rescinnamnine was clinically a less potent alkaloid than Reserpine and lowering of blood pressure was not significant.

1.7: Medicinal uses:
1.7.1: Ethnobotanical uses: (Dey and De, 2011).
1. Freshly ground leaves when applied to the toes could serve as an antidote for snake poison.
2. The mentally challenged person is relieved from insanity by consumption of root.
3. This plant was found to be used very commonly by tribes indicating the authenticity of their usefulness. The inhabitants of Makassar use the petioles as an antidote for infusion.
4. Decoction and extracts of the roots are employed to increase uterine contractions for expulsion of fetus.
5. It is also useful against painful affections of bowels, diarrhea, dysentery, cholera and colic.
6. Ethno medical use of this plant to treat circulatory disorders.
7. Young shoot extract of this plant (ca 10mL) is given three times daily to cure pneumonia in early stage by the Meche People of Jhapa District, Eastern Nepal.
8. *Rauwolfia serpentina* root and leaf paste to make pills and sun dried used against malarial fever.
9. The root juice is used during the time of liver pain.
10. The fresh leaf juices are used to prevent eye inflammation.
11. Rural people of Kanyakumari district, India, use the decoction of roots during labour and juice of leaves for removal of opacities of the eye cornea.
12. Roots are chewed for stomach pain and fever by Khamptis of Arunachal Pradesh, India.
12. Garo tribe inhabiting the Madhupur forest region of Bangladesh uses this plant to treat malaria and spleen diseases.
13. A paste of root and black pepper is administered for malaria (Dey and De, 2010).
14. High blood pressure:

   The Rauwolfia herb is the best remedy for high Blood Pressure and it has been adapted by medical fraternity in most countries. Those alkaloids which have a direct effect on hypertension have been isolated and are widely used by the practitioners of modern medicine.

15. In insanity:

   The Rauwolfia plant is highly beneficial in treating insanity. One gram of powdered root can be taken twice a day with 250 ml of goat’s milk, sweetened with sugar candy. It is unsuitable for those with a low blood pressure, depressed and hypotensive patients.
16: In insomnia:

   Rauwolfia is a well known remedy in treating insomnia because of its sedative properties. The very first dose of Rauwolfia enables the patient of a phlegmatic and gouty nature to go to sleep. About 0.6 to 1.25
grams of the powder of its root is mixed with some scented vehicle and taken. It is non-stimulating and should be given in doses of 0.25 grams to the patient at bedtime for sound sleep.

17: In Hysteria:

Rauwolfia is useful in treating hysteria. One gram of powdered root can be administered thrice with milk. Treatment should be continued till a complete cure is obtained.

18: In itching skin:

It relieves itching in urticaria. One gram of powdered root can be taken with water.

19: Prostate cancer:

Prostate cancer is considered to be major causes of cancer-related deaths among men. Modern techniques such as chemotherapy and radiotherapy have not provided significant survival benefits to patients with prostate cancer. Natural products have proved to be a major resource for identification of bioactive compounds used in the treatment of a variety of ailments and diseases, including cancer as compared to chemotherapy and radiotherapy. Various parts of this plant have been used as a traditional medicine for centuries to treat a variety of ailments including fever, general weakness, intestinal diseases, liver problems and mental disorders. Extracts from the root bark of this plant are enriched with compounds of β-carboline alkaloid family of which the main constituent is alstonine. This compound has been previously reported to reduce tumour cell growth in mice inoculated with YC8 lymphoma cells or Ehrlich ascetic cells. The plant extract has anti-prostate cancer activity in both *in vitro* and *in vivo* model systems which, based upon analyses of gene expression patterns of treated prostate cancer cells, may be modulated by its effects on DNA damage and cell cycle control signaling pathways.
20. Snake, insect and animal bite:

There are many folk-lore’s about this plant. One of which is that a mongoose would first chew upon its leaves to gain power before combating a cobra. According to another, it’s freshly ground leaves when applied to the toes could serve as an antidote for snake poison. In case of snake-bite, juice extracted from leaves taken twice a day for three days. 15 g of roots along with roots of Cassia tora and Holarrhena pubescens paste applied twice a day for two days by Khamptis of Arunachal Pradesh, India. The roots and leaves of this plant are crushed with milk and made into a paste and used internally and externally on the affected area in case of snakebite by the people of Bhadra wildlife sanctuary in Karnataka ((Sen et. al., 2008 and Parinitha et. al., 2004).

21. By action on the vasomotor centre, it leads to generalized vasodilatation, with a lowering of blood pressure.

22. By depressant action on the cerebral canters, it soothes the general nervous system.

23. It stimulates the bronchial musculature.

1.7.2: Therapeutic Uses:

Sarpagandha has been employed therapeutically from centuries in India for the relief of various central nervous system disorders, both psychic and motor. These include anxiety states, excitement, maniacal behavior associated with psychosis, schizophrenia, insanity, insomnia and epilepsy. Root is bitter tonic, hypnotic, sedative, specific for insanity, reduces blood pressure. It is a remedy for painful affections of the bowels. Extract of roots is used for the treatment of intestinal disorders, particularly diarrhea and dysentery. Root stimulates urine contraction and are used in child birth in difficult cases. Decoction of roots is employed in
labors to increase urine contractions. Leaves are bitter stimulant uterus, nutritive, anthelmintic. (Joshi, 2000).

1.8. Cultivation: (Kavita, 2005 and Pandey and Chadha, 1993)
1.8.1: Soil:

The Sarpagandha plant can be grown in different variety of soils, from sandy alluvial loam to red lateritic loam of stiff dark loam in its natural habitat. It prefers clay loam with a large percentage of humus. It does not grow well having pH 8 or above of soil. The favourable pH for growing this crop is from 4.6 to 6.2. Generally, the plant produces thicker roots in black, stiff loam soils than in heavy clayey or sandy soil. The large quantities of sand containing in soil, reduce the growth of the plants and become more susceptible to root and leaf diseases.

1.8.2: Climate:

Rauwolfia serpentina can be grown under a wide range of climate conditions. It flourished in hot, humid conditions and can be grown both in the sun and in partial shade. In its natural habitat, the plant thrives under the shade of forest trees. It prefers a tropical or sub-tropical belt having the benefit of monsoon rains, preferably the South-west. Localities in the Deccan Peninsula, which are with more equitable climate throughout the year as compared to the sub-Himalayan tracts are said to be more suited for a profitable cultivation of this plant. A climate with a temperature range of 10-30% seems to be well suited for this plant. The best areas for its growth are those which combine high rainfall with properly drained soil. Although it has been reported to grow naturally where rainfall is about 250 cm annually, it grows well in areas with a rainfall of even 500 cm or more. In low rainfall areas, the plant can be successfully cultivated, if irrigation is available during the drier months. Though the plant seems to be sensitive to water-logging, it can tolerate
water for 2 to 3 days without too much damage. The plant sheds its leaves during the cold months in localities with severe winters. Frost kills the top tender, green twigs only and fresh shoots sprout up with the advent of spring from the thicker shoots which can withstand the frost.

1.8.3: Propagation:

Rauwolfia can be propagated by seeds and also by vegetative means like root cutting, root stumps, stem cuttings.

1.8.3.1: Seed propagation:

Commercially, the plant is usually propagated by seeds, irregular and very low percentage of germination of seeds is the main difficulty in the propagation of Rauwolfia. The percentage of germination of seeds is quite variable, ranging from 10-60%. This is partly attributed to the adverse influence of the stony endocarp. Another serious factor is the absence of embryos, though the fruits may appear perfectly normal externally. This may possibly be an effect of parthenocarpy.

The rate of germination however depends on the percentage of fully matured heavy seeds in a particular lot. Fresh seeds, collected from ripe fruits and immediately sown, show a higher percentage of germination (58-74%). The ripe seeds collected from the beginning of June to the end of October or even November and stored in airtight bins, retained their viability for about 6 months. The viability of the seeds decreased drastically with the increase in the interval of time between collection and sowing. The germination rate of the seed also differs under varying agro climatic conditions.

The seeds should be usually collected from September-February. Fruits mature between July-November. Only a few fruits ripen at a time and, if they are not collected immediately, they shed and are lost.
Therefore, the collection of ripe fruits twice a week is necessary. While this is easy in case of plantation, collection from plants growing in the wild is both laborious and costly owing to their scattered distribution over large areas.

The fruits, after collection, are freed from their pulpy covering by rubbing them against old gunny bags or on rough flooring. The cleaned seeds are thoroughly dried in the sun and store in dry places or in airtight containers; a yield of 100-120 kg of clean seeds can be obtained from one hectare of a 3 year old plantation. An adequate supply or seeds can also be obtained by raising the plants vegetative in compact areas, preferably by using stem-cuttings.

The sowing of the seeds directly in the field has not been successful and, hence seedling are raised in the nursery and transplanted into the field. The nursery should ideally be located in partially shaded areas with irrigation facilities. The land is cleared of weeds and ploughed to a depth of 30 cm. Raised beds are made, which should contain one third quantity of well rotted FYM and two thirds of fine soil. Seeds are sown in the middle of May. The seeds should be soaked in water overnight and the light seeds which float discarded. The seeds can be treated with Thiram at the rate of 3g/kg of seeds. About 5.5 kg of seeds sown in a 500 m2 area will yield seedlings sufficient to plant one hectare. The germination is gradual and the growth of the seedlings is slow. Germination starts after 15-20 days and continues up to 40-45 days after sowing. The nursery should be kept moist throughout the germination period.
Transplanting:

The seedlings of 40-50 days which have 4-6 leaves are transplanted. The seedlings are carefully dug out and the top root should be cut. They are then dipped in a 0.1% soil-borne fungus causing damping off disease. Well rotted FYM at 25-30t/ha is added during land preparation. The field is then divided into small plots for irrigation. About 15 cm deep furrows are dug at a distance of 45 cm. The seedlings are transplanted into the furrows, by making holes large enough to receive the seedlings along with the accompanying clump of earth. A sampling of 30 cm between the plants should be maintained. The seedlings are buried up to the first pair of leaves and soil around them is lightly pressed. Irrigation after transplanting is essential, and should be continued at regular intervals until the seedlings are established.
1.8.3.2: Vegetative propagation:

As collection of seeds from wild sources is both laborious and costly, vegetative propagation by root or shoot cuttings has been advocated for raising plantation which also quickly multiply the genetically superior clones.

1.8.3.2.1: By root cuttings:

Large tap roots with a few filly form lateral secondary rootlets are used. Cuttings of 2.5-5.0 cm length are planted in holes, at the beginning of the monsoon and are almost completed covered with earth, leaving only 1 cm above the surface. Nearly 50% of the root-cuttings sprout in about a month.

Trials have shown that under irrigated conditions, root cuttings of about 0.25 cm diameter planted during March-June gives a 50-80% success rate. About 100 kg of root cuttings are required to plan 1 ha. The high percentage of success obtained by root cutting makes it more preferable than propagation by seeds. However, the recovery of roots from them has been found to be not high as in plants raised from seeds.

![Fig.4: Roots of Rauwolfia serpentina](image)
1.8.3.2.2: By root-stumps:

About 5 cm of root with a portion of the stem above the collar region is used for propagation has also been attempted. This method gives about 90-95% success, sometimes even 100% such plants transplanted in May-July into irrigated fields become well established by the end of September. This method has its limitation as only one plant can be raised from a single stump.

1.8.3.2.3: By stem cuttings:

Stem cuttings taken from woody twigs have also been tried as a source of propagation. Hard wood cuttings have been found better than soft wood cuttings. Cuttings of 15-22 cm length, with three internodes are the most suitable. Nearly 60-100% of rooting is obtained by treating hardwood cuttings with indole acetic acid solution of 30ppm for 12 hrs and the treated cuttings root within 15 days. Stem cuttings planted in the nursery during the early monsoon (June) and kept moist until they sprout that gives about 40-65% success rate. Such cuttings, though start sprouting 3-4 days after planting, naturally strike roots mostly after about 75 days. Stem cuttings have been found less satisfactory than root-cuttings, since many of them do not root easily.

Fig. 5: Stem of *Rauwolfia serpentina*
1.8.4: **Manuring:**

The use of organic manure, mould and compost has been recommended to increase the quantity of nutrients in the soil and improve the drainage. The plants responded better to chemical fertilizers than to organic manures. Nitrogenous fertilizers induce more vegetative growth, followed by organic manure. Nitrogen seems to have a stunting effect on the root. But the combination of nitrogen either with FYM or phosphates results in better root growth than nitrogen alone.

Application of phosphates induces more growth of thick as well as thin roots. Since good manure is in short supply and uneconomical artificial fertilizers should, therefore, be preferably used. It is better to apply 25-30 t of well-rotted FYM at the time of land preparation and 10 kg N60 kg P2O5 and 30 kg K2O. ha as a basal dose. Later two equal doses of N, each of 10 kg./ha in moist soil is given at 50 days and 170 days after planting.

1.8.5: **Irrigation:**

The crop may be irrigated fortnightly in the hot dry season and about once a month in winter. The crop can be cultivated under rain fed conditions also, but the yield is considerably poorer.

1.8.6: **Weeding and intercultural practices:**

In order to maintain the satisfactory development of roots about 2 weeding are necessary during the monsoon and one hoeing at the end of the growing season (December). This may be done in large plantations using a tractor-drawn cultivator Viz. cheaper than manual labor. Hoeing by means of a tractor-drawn wheel-hoe is the most economical.
1.8.7: **Intercropping:**

It is possible to grow intercrops Rauwolfia plantation, particularly where good irrigation facilities are available. It is reported that although the yield of roots was higher under monoculture, the net returns were highest when Rauwolfia was intercropped with soybeans and onions or soybeans or garlic.

1.8.8: **Harvesting and processing:**

The roots of exploitable size are generally collected 2-3 years after planting i.e. from 18 months onward. It is reported that roots dug out in winter (December). When the plants have shed their leaves are richer in total content of alkaloids than the roots harvested in August. A light irrigation should be given in advance to facilitate easy digging of roots. The roots may be dug out carefully from the subsoil, manually or by using a mould board plough.

Care should be taken to keep the root bark intact as the bark constitutes 40-56% of the whole root and has a higher alkaloid content. The roots are freed from the adhering soil, washed if necessary and thoroughly air-dried till they become brittle and are usually packed in gunny bags. Roots containing 8% moisture keep better. They are stored in a cool, dry place to prevent mould.

When the roots are to be stored for a short period, they may be packed in gunny bags or polythene bags, pending disposal. But these must be periodically turned, to protect them from mould which is reported to reduce the total alkaloid content ranging from 5-25%. Under the present system, only tap-roots are selected for processing. It has been observed
the rootlets are also rich in alkaloids, so these should be included in the material.

Though *R. serpentina* can be propagated by various methods, the optimum yield of roots (including thick, thin and fibrous) is obtained when the propagation is done by seeds. The yield of fresh roots per plant varies widely from 0.1-4 kg. with a spacing of 60×30 cm, the total yield of roots in the case of plants raised from seeds works out to about 1175 kg./ha on air-dried basis, compared with 175 kg./ha in the case of plants raised from stem-cuttings. A yield of 2200 kg /ha of air dried roots has been obtained from a 2 year old plantation, under irrigated conditions on sandy, clay loam soil.

1.8.9: **YIELDS:**

The optimum yield of roots is obtained by propagation through seeds. The yield of fresh roots per plant varies widely from 1-4 kg. the total yield of roots in the case of plants raised from seeds is about 1175 kg/ha on air dried bases as compare to 175 kg/ha in case of plants raised from stem cuttings and 345 kg/ha in case of root cuttings.

1.8.10: **Economics of cultivation**

1. Cost of seed @ Rs. 2000 x 5 kg 10000
2. Nursery raising 1500
3. Land preparation 6000
4. Organic manure & application cost 10000
5. Cost of transplanting 2500
6. For Irrigation L.S 2000
7. Weeding, hoeing and light earthling up 6000
8. Plant protection 1500
9. Maintenance cost for 20 months 2500
8. Harvesting & carrying, processing etc 8000
Total Rs 50 000
Yield — 2000 Kg. Dry roots at 30 months
Gross return @ Rs. 80/- per Kg. (2000 x 80) ....... . . Rs 160000
Total cost of production.......... Rs. 50,000
Net return from 30 months cycle crop harvesting Rs. 1, 10,000

1.11: Conservation status:

*Rauwolfia serpentina* is threatened with extinction in India due to indiscriminate collection and over exploitation of natural resources for commercial purposes to meet the requirement of the pharmaceutical industry, coupled with limited cultivation. Collection and conservation of this plant was carried out from south Karnataka and Western Ghats of India. The genetic erosion has affected the species greatly and populations left in India have very poor alkaloid content. It was found to be endangered in Southern Western Ghats of India. It has been categorized as globally endangered. This species are endangered and threatened in Kanyakumari district, India. The plant was described as critically endangered in the Northeast India. (Dey and De, 2010)

Sarpagandha medicinal plant is threatened with extinction in India due to indiscriminate collection and over exploitation of natural resources for commercial purposes to meet the requirements of the pharmaceutical industry, coupled with limited cultivation. In view of this, there is need in *Vitro* and *in Vivo* for conservation of this important threatened plant species. Sharma and Chande, (1992).
1.12: Disease and Pests:

1.12.1: Diseases:

*Rauwolfia Serpentina* is susceptible to cause various diseases. The different fungi are responsible for infection to the foliage and roots of Sarpagandha viz. responsible for decrease in biochemical productivity. In India, indole biochemical alkaloids are used to regulate heart beat of human beings. Such Indole alkaloids are synthesized in the root of *Rauwolfia serpentina*. Thus, plant alkaloids contents are reduced by fungal foliar and root diseases.

The different diseases of Sarpagandha (*Rauwolfia serpentina*) are reported as follows. (Mukerji and Bhasin, 1986), Mehrotra and Thapar, (1990), Purohit and Vyas, 2007 and Dey and De, 2010).

1. Leaf spot - *Alternaria alternata* (Fr.) Keissler
2. Leaf spot - *Cercospora rauvolfiae* (Chupp & Muller)
3. Leaf spot - *Cercospora serpentina* (Pandotra & Hussain)
4. Leaf spot - *Colletorichum capsici* (Syd.) Butler & Bisby
5. Anthracnose - *Colletotrichum gloeosporioides* (Penz.)
6. Target spot - *Corynespora cassicola* (Berk. &Curt.)
7. Leaf spot - *Curvularia lunata* (Wakker) Boediiin
8. Leaf spot - *Epicoccum nigrum* (Link.)
9. Wilt - *Fusarium oxysporum* (Schlecht)
10. Wilt - *Fusarium rauvolfi* (Janardhanan etal)
11. Powdery mildew - *Leveillula laurica* (Lev.) Aranud
12. Root rot - *Macrophomina phaseolina* (Tassi) Goid


14. Leaf spot - *Pellicularia filamentosa* (Pat.) Rogers

15. Die back - *Colletotrichum dematium*

16. Leaf blight and Bud rot - *Alternaria tenuis*

17. Leaf spot and blight - *Rhizoctonia solani*

### 1.12.1.1: Details of Diseases:

1. **Root rot:**

   Root rots disease on *Rauwolfia serpentina* caused by *Macrophomina phaseolina*.

2. **Leaf spot:**

   Leaf spot is caused by *Cercospora rauvolfiae* Chupp & Muller manifests as spots of dark brown color on the upper surface of the leaf and as yellowish brown on the lower surface. Olivaceous effuse fructification is produced on the lower leaf surface. The affected leaves turn yellow, become dry and subsequently fall off, resulting in defoliation.

3. **Leaf blight and bud rot:**

   *Alternaria tenuis* attacks the leaves of *Rauwolfia Serpentina*, showing minute brownish, dark colored circular spots with yellowish margin on the ventral side of the leaves; these enlarge to prominent dark
brown circular lesions. Diseased leaves finally turn brown and fall off. The fungus also affects the flowers and fruits.

The disease can be controlled by spraying with Diathane M-5(0.3%). it can be also controlled by seed treatment with fungicides.

4. Mosaic:

The mosaic disease is common disease on Rauwolfia Serpentina. The primary symptom comprises vein bending and gradual yellowing of leaves. Later, the leaf curls from the margin to the midrib on the abaxial surface and ultimately the leaf drops off.

5. Root knot:

Root knot appears as galls of various sizes, up to 8 mm in diameter, covering the root system. Stunted growth of plants, etiolating and decrease in leaf size are the symptoms in the aerial portion. The galls on examination show mites, various soil fungi and nematodes (Heterodora Sp.) Generally, brown clay soils supporting the natural growth of R. Serpentina, are found to be infested with nematodes, while dark clay soils are free from them.

6. Leaf spot:

Cercospora rauvolfiae causes small dark brown spots on the upper surface of the leaf and yellowish brown on the lower surface. These spots subsequently merge and cause yellowing and drying of the affected leaves resulting in defoliation. Control can be done by spraying Dithane Z-78 or Dithane M-45. Mycosphaerella rauwolfia also causes grayish black small spots which are oval or round in shape. The lowest leaves are attacked first and the disease progresses upwards.

7. Target leaf spot:

Corynespora cassiicola causes dark brown spots on the upper and yellowish brown spots on the lower surface of the leaves throughout the growing season. These spots gradually enlarge into circular spots, 2-20
mm in diameter with concentric zones due to which disease is named as "target spot". Heavily infected leaves turn yellow and are shed prematurely.

Captain sprayed as 0.25 per cent solution in water, in early June before monsoon and repeated at monthly intervals until November is most effective in controlling the disease.

8. Leaf blotch:

This is caused by *Cercospora serpitinae* purple, mud-colored blotches appear on the ventral surface of the leaf. Disease occurs as a small dot increasing to a circular patch, which later coalesces to an irregular form. In week’s time the affected portion become maroon in color and the whole leaf gradually dies. Spray of captan and Dithane Z-78 or M-45 may prove useful in controlling this disease.

9. Anthracnose:

*Colletotrichum gleosporioides* causes the disease and appears as numerous tiny spots of the aecervuli scattered all over but mainly confined to the upper leaf surface. An infected spot enlarge into circular patches and invades the surrounding tissues. Several such patches coalesce and cause drying of the lamina resulting in defoliation. Every infected spot is an aecervulur packed with spores. Cutting and burning the infected shoots, avoiding overhead watering which causes easy dissemination of spores by water splashes, and using dithiocarbonates such as fermate, Dithane and terlate in place of Bordeaux mixture, have been recommended for controlling the disease.

10. Die back:

*Colletotrichum dematium* causes numerous spots bearing aecervuli scattered all over the surface of twigs leaves and flowers. Sometimes smaller lesions coalesce to form large and circular necrotic patches resulting in complete destruction of leaf lamina and subsequent
defoliation. In high humidity conditions the disease aggravates. The Spraying Dithane Z-78 (0.2%) at the advanced stage of infection subsequent to pruning of the affected parts controls partially the spread of disease. (Varadarajan, 1964)

1.12.2: Pests:

Several pests are also reported on Rauwolfia serpentina which causes extensive damage to the plant.

The caterpillars roll the leaves, harbour in the rolls and feed on green matter causing extensive damage to the foliage.

2. *Deilephila nerii* (sphingid moth).
The young leaves are attacked by the caterpillars which feed on them causing defoliation of the plant. The incidence of pest is severe during winter. Dusting 5% dipterex or 2% tolidol dust gives effective control.

3. *Scarabacide sp.* (Grub).
The pest profusely attacks the main roots of the seedlings during June resulting in their drying up. Mixing BHC (10%) with the soil at the time of preparation of land can control the grubs to some extent. The other pests reported to cause damage to the crop are *Diaphania nilgirica* (moth), *Anomala polita* (Cockchafer grub), and *Saissetia nigra* (black bug).

1.13: *Macrophomina phaseolina* (Tassi) Goid:

*Macrophomina phaseolina* is a soil-borne fungal pathogen, infecting more than 400 plant species Under favorable conditions the fungus causes many diseases like damping off, seedling blight, collar rot, stem rot, charcoal rot and root rot in various economically important crop. The infectious hyphae enter into the plant through root epidermal
cells or wounds and mycelium penetrates into the root epidermis and limited the intercellular spaces of the cortex of primary roots, as a result, adjacent cells collapse and heavily infected plantlets may die due to the production of fungal toxins e.g. phaseolinone. Charcoal rot reduced the crop yield up to 100% and 23-64% grain yield under favorable conditions especially in arid regions of the world. The fungus can remain viable for more than 4 years in soil and crop residue as sclerotia. Under favorable conditions, hyphae germinate from the sclerotia and infect the roots and/or stem of the host plant by penetrating in the plant cell wall through mechanical pressure and/or chemical softening. The disease progresses from leaf yellowing to wilting and ultimately plant death. The disease development is favoured with high temperature (30-35°C) followed by moisture stress but in case of jute and corn it prefers the hot, moist and humid condition. It is difficult to control *M. phaseolina* due to its thick-walled resistant hyphal mat called sclerotia persistence in the soil and plant debris. Recently, there has been a worldwide report in increased incidence of the pathogen on diverse crop species which could reflect a wider appreciation of the importance of this disease to crop production in drought prone regions.

1.13.1: Classification of *Macrophomina phaseolina*.

Domain: Eukaryota

Kingdom: Fungi

Division: Ascomycota

Sub division: Pezizomycotina

Class: Dothidiomycetes

Order: Botryosphaeriales

Family: Botryosphaeriaceae

Genus: Macrophomina

Species: phaseolina
1.13.2: Importance of the topic:

This fungal pathogen cause severe crop losses. In soybean growing regions Charcoal rot is a serious problem. It causes more annual economic losses in the top-ten i.e. United States, Brazil, Argentina, China, India, Paraguay, Canada, Indonesia, Bolivia and Italy soybean-producing countries. Only in the United States, a total yield loss of $173.80 million during 2002. The incidence of *M. phaseolina* in sorghum fields was reported up to 70% in Somalia. Yield losses due to *M. phaseolina* infection have been estimated up to 57% in sesame (*Sesamum indicum* L.) at 40% disease severity. In Bangladesh, the fiber yield of jute can be reduced by 30% due to this fungus.

Among these diseases, the root rot disease caused by *Macrophomina phaseolina* was found to be severe in Sarpagandha. The valuable reserpine chemical content may be reduced due to this disease, hence selected for detailed investigation. The aims and objectives of the present investigation are as follow.

1. Collection of healthy and diseased plant roots from various areas.
2. Isolation, purification and pathogenicity of the pathogen.
3. Identification of the pathogen.
4. Effect of phytoextracts on growth of *Macrophomina phaseolina*.
5. Effect of biocontrol agents on growth of *Macrophomina phaseolina*.