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# Influence of propagation techniques and harvesting time on root yield and alkaloid contents of *Rauvolfia serpentina*

**A. K. Pandey*, A. K. Mandal**

Tropical Forest Research Institute, P. O. RFRC, Mandla Road, Jabalpur - 482021. India

*Rauvolfia serpentina* (Fam. Apocynaceae) is an important medicinal plant. Mass scale collection of this plant from natural habitats has lead to a depletion of plant resources. In order to reduce the pressure on natural resources a profitable cultivation technique to obtain higher root yield and alkaloid content is essential. A field experiment was carried out to find out the influence of propagation techniques and harvesting time on root yield and total alkaloid content of the crop under tropical climatic conditions. The crop was propagated by transplanting seedlings raised through seed and vegetatively by root and stem cuttings. Results revealed that harvesting time had pronounced effect on quality and yield of the herb. However, propagation techniques did not have significant effect on total alkaloid content. Highest root yield and alkaloid content was obtained in the crop raised though seeds harvested after 18 months of planting. December was found ideal time to harvest the crop as it yielded higher alkaloid content.

**Key words:** *Rauvolfia serpentina*, propagation, harvesting time, alkaloid content.

## 1. Introduction

*Rauvolfia serpentina* (L.) Benth. ex. Kurtz. is an endangered woody perennial of the family Apocynaceae, found widely distributed in the Himalayan foothills and Peninsular India. The plant is medicinally important in the treatment of cardiovascular diseases, hypertension and various psychiatric diseases (Rand and Jurevics 1977; Sahu, 1983). Interestingly, its roots were long used in India for treating mental illness and snakebite, known to medicine man and peasants as the "Insanity herb" or "snake root" or "Sarpagandha". The alkaloids are effective against snake bites and scorpion stings (Anonymous 1969). The plant is reported to contain various indole group alkaloids that are localized in the roots (Phillipson and Zenk, 1980). More than 50 alkaloids have been reported from *R. serpentina*. The most important alkaloids isolated from the roots are reserpine, ajmaline, ajmalicine, ajmalinine, serpentine, alloyohimbine, chandrine, deserpidine, isoajmaline, yohimbine,

* Corresponding author
Email: akpandey10@rediffmail.com

r-yohimbine, isoyohimbine, 11-methoxy-delta-yohimbine, methylreserpate, neoajmaline, papaverine, corynanthine, isorauhimbine, 3-epi-alpha-yohimbine, raunatine, rauvolfinine, rauwolscine, reserpiline, rescinnamine, reserpinine, reserpoxidine, sarpagine, serpinine, and serpentinine (Virmani *et al*., 1992). Rauvolfia roots are reported to contain 0.7-3% of total alkaloids in the dry mass and the amount varies depending upon the time and source of collection (Kokate *et al*., 1998). Chatterjee (1956) studied the seasonal variation in total alkaloid content of *R.canescens*. The highest alkaloid content (1.69 %) was found during the period from October to January. Sobte, *et al*. (1951) reported that the yield of roots and alkaloid content increases with the age of the plant. Rajagopalan (1954) reported that *R. serpentina* roots collected from Western Ghats of Bombay, Madras and Travancore-Cochin contained alkaloids ranging from 1.17 to 1.73 percent. Biswas (1956) in West Bengal and Ahluwalia (1963) in Saurastra also reported differences in alkaloid content of roots. The alkaloid content in the plants grown in Bangaldesh was 1.43 percent (Ahmad *et al*., 2002).

It grows well in tropical to subtropical climate receiving heavy rains between June and August. The areas having more equable climatic variations seem to be more suited than the areas having higher climatic variations. It prefers soil with plenty of humus and rich in nitrogenous and organic matter with good drainage. Alkaline soils are not suitable for commercial cultivation. The plant sheds its leaves during winter.

In pharmaceutical industries, roots of Rauvolfia and its products are in great demand. Even though the chemical synthesis of reserpine is possible, it costs more than extracting it from natural resources (Farooqi and Sreeramu, 2001). Mass scale collection of this plant from natural habitats has lead to depletion of plant resources. The availability of *R.serpentina* has been decreased in the wild to the extent that it has been included under the category of endangered plants, and also included in the list of the items banned for export. There is an urgent need to conserve this important endangered medicinal plant. To meet the increasing demand of *R.serpentina* roots we need a farmers friendly cultivation package by which good quality of roots should be obtained on sustainable basis. The farmers/growers are practicing different propagation methods for its cultivation. There is need to find out the most suitable method of cultivation in terms of root yield and alkaloid content. Keeping the above facts into consideration, the present study was conducted to determine the influence of different propagation techniques and harvesting time on root quality and productivity of *R.serpentina*.

## 2. Materials and Methods

The experiments were conducted at Tropical Forest Research Institute, Jabalpur India and its centre at Chhindwara. The soil of the experimental field was sandy loam having organic carbon 0.45 %, available N 175.32 kg/ha, available P 26.45 kg/ha and exchangeable K 215 kg/ha with pH 7.12. *R.serpentina* crop was cultivated by planting seedlings/plantlets raised through seeds, root and stem cuttings as per existing agronomical practices (Pandey *et al*., 2001).

### *2.1 Propagation*

**Land Preparation:** The land was ploughed deep in April May and left for weathering. After pre monsoon showers FYM (40 tonnes/ha) was added followed by ploughing and two cross harrowing to break the clogs. The land was finally dressed by planking and the beds were laid out.

**Fig 1.** *Rauvolfia serpentina* (Sarpgandha) plant

Fig 2. *Rauvolfia serpentina* crop growing in the field

Fig 3. *Rauvolfia serpentina* (Sarpgandha) roots

**Table 1.** Growth characteristics of *R. serpentina* plants raised by different propagation methods

| **Treatments** | **Growth characteristics of *R. serpentina* plants** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **6 months** | | | **12 months** | | | **18 months** | | |
| | **Height (cm)** | **Collar diameter (mm)** | **Number of branches** | **Height (cm)** | **Collar diameter (mm)** | **Number of branches** | **Height (cm)** | **Collar diameter (mm)** | **Number of branches** |
| Seeds | 22.33 | 4.24 | 2.56 | 32.67 | 5.20 | 3.52 | 45.67 | 7.42 | 5.12 |
| Root cuttings | 18.67 | 4.25 | 2.25 | 32.33 | 5.78 | 3.84 | 42.33 | 8.71 | 4.82 |
| Stem cuttings | 17.00 | 4.50 | 2.15 | 30.00 | 9.14 | 3.25 | 40.38 | 9.04 | 4.54 |
| CD (5%) | NS | NS | NS | NS | NS | NS | NS | NS | NS |

**Table 2.** Root biomass and alkaloid content of *R. serpentina* roots raised by different propagation methods.

| Treatments | Harvesting time of *R. serpentina* roots | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 6 months | | | 12 months | | | 18 months | | |
| | Fresh weight (gms) | Dry weight (gms) | Total alkaloid content (%) | Fresh weight (gms) | Dry weight (gms) | Total alkaloid content (%) | Fresh weight (gms) | Dry weight (gms) | Total alkaloid content (%) |
| Seeds | 20.33 | 7.67 | 0.41 | 41.46 | 13.52 | 0.69 | 58.73 | 25.23 | 1.97 |
| Root cuttings | 18.33 | 6.27 | 0.35 | 40.05 | 12.31 | 0.59 | 48.33 | 21.48 | 1.71 |
| Stem cuttings | 18.38 | 6.31 | 0.38 | 37.33 | 11.30 | 0.56 | 47.66 | 19.77 | 1.63 |
| CD (5%) | NS | NS | NS | NS | NS | NS | 3.07 | NS | NS |

**Nursery:** Nursery was prepared in partially shaded area with adequate irrigation facilities. One-third of well matured FYM, and two-third amount of sand and soil was made the medium of beds. Each bed was 1 metre wide, 15x20 cm high and of convenient length. About 5-8 kg seeds are required for planting in one-hectare area. Fresh seeds were preferred for sowing as their viability lasts for only 6-8 months. It has been observed that the seeds stored for more than a year usually failed to germinate. Therefore it is essential that the seeds collected between September to December should be used for planting in the next season. Seeds were soaked in water for 24 hours before sowing. Only heavy seeds were selected by floating them in water. Presoaked seeds were sown in nursery beds at 6-8 cm apart in rows in shallow furrows during 1st week of May. The furrows were then covered with a fine mixture of soil and FYM. Beds were kept moist by light watering. Germination started after 20-25 days and continued up to 30 to 40 days. Germination percentage varied from 15-55 %. It was also propagated by vegetative means using stem and root cuttings.

**Root cuttings:** 3-5 cm long root cuttings were planted 1-2 cm deep in the beds filled with sand in 1st week of May. The cuttings sprouted within three weeks. Success percent was 40-85 and around 100 Kg of root cuttings are required for 1 ha area. The sprouting of cutting was directly dependent on time of planting. May-June was found the best time for raising the plantlets though root cuttings.

**Stem cuttings:** 15-20 cm long stem cuttings with 3-4 nodes were planted in the nursery in May-June. The cuttings were dipped in 100 ppm solution of IBA before planting. The lower end of the cuttings was planted in the bed. However, the opposite end of cuttings was sealed by wax. The beds were kept moist by giving light irrigation daily. The success percent was from 40-65 %.

Seedlings/plantlets were ready by 1st week of July for transplanting. The seedlings were transplanted in the field in the second week of July at 45 x 45 cm. Light irrigation was given immediately after planting. In monsoon season the crop was irrigated as and when required.

**Maintenance of crop:** The seedlings generally did well and gaps, if any, were filled by fresh planting. It is a one-and-a-half to 2 year crop and need regular irrigation, weeding and hoeing. The crop was given weekly irrigation during May and June, fortnightly irrigation from January to April and monthly during autumn. 5 weeding and hoeing were done during its entire

growing period. No chemical fertilizer was used for cultivation because at present organically grown medicinal plants are in demand.

**Harvesting:** The crop was harvested after 18 months of planting i.e. in the month of December when the plant sheds its leaves. 8-10 days prior to uprooting the crop was irrigated and the above ground foliage was cut and the roots were uprooted. The roots were cleaned, washed and dried in shade. Care was taken not to damage outer bark of roots while uprooting as it contains maximum amount of alkaloid.

**Determination of fresh and dry weight:** The roots were collected from the nursery and washed under tap water to remove soil. Then they were placed under a fan for drying and weighed with an electronic balance. After fresh weight determination the roots were placed on Petri plates and dried in shade. Dried root weight was recorded carefully with an electronic balance.

**Alkaloid extraction and analysis:** The dried roots were extracted for total alkaloid contents (Ahmad *et al*., 2002). The dried root powder was extracted with rectified spirit for 10 hours. The solvent was evaporated under reduced pressure in a rotary evaporator to obtain a brownish residue. The residue was suspended in water and extracted with petroleum ether (50 mlx3) using separating funnel to remove pigments and fatty substances. The organic solvents were combined and evaporated under reduced pressure to obtain green masses. Then the aqueous layer was separated and extracted with chloroform (50 mlx3) and the solvent was evaporated under reduced pressure to obtain neutral chloroform extracts. The residual aqueous layer was made acidic (pH 3.0) by adding 0.1 N HCl and extracted with chloroform (50 mlx3) and the solvent was distilled under reduced pressure to obtain acidic chloroform extract. All acidic chloroform extract was made alkaline by adding $NH_4OH$ solution and again extracted with chloroform (50 mlx3). Chloroform extract was tested for alkaloid using Dragendroffs reagent. The content was evaporated under vacuum and the weight was recorded as total alkaloid content.

## 3. Results and Discussion

Growth characteristics (height, collar diameter and number of branches) of *R. serpentina* plants grown through different propagation techniques were recorded and depicted in Table 1. Height of the *R. serpentina* plants after 18 months was 45.67 cm in plants raised through seeds, 42.33 cm in the plants raised through root cuttings and 40.38 cm in the plants raised through stem cuttings. The collar diameter of 18 months old plants raised through seeds, root cuttings and stem cuttings were 7.42 mm, 8.71 mm and 9.04 mm respectively. There was not much difference in the growth pattern of the plants raised through various propagation methods. However, the plants raised through vegetative means (root and stem cuttings) started flowering very early i.e. two months after planting. Fresh and dry root weight was determined from the crop raised through various propagation methods. The roots were harvested from the crop at regular intervals i.e. after every six months. The roots were dried in the shade, and 55-60 percent of their weight was lost during drying. Data on fresh and dry weight of the roots are presented in Table 2. Fresh root biomass of the roots obtained from 18 months crop raised through seeds was maximum (58.73 gm) followed by crop raised through root cuttings (48.33 gm) and stem cuttings (47.66 gm). However, the dry weight of the roots of 18 months old crop raised through seeds, root cuttings and stem cuttings were 25.23 gm, 21.48 gm and 19.77 gm respectively. Data revealed that crop raised through seeds yielded more roots in comparison to crop raised by vegetative means i.e. root and stem cuttings. *R.serpentina* roots were harvested at various stages of maturity. Chemical analysis showed that there was not

much difference in the total alkaloid content in the crops propagated through seeds, root and stem cuttings. There was difference in the root yield and alkaloid content in the roots harvested after 6 and 18 months of planting. The roots obtained from the crop raised through seeds and harvested after 18 months of planting possesses higher alkaloid content (1.97%) compared to roots obtained by the plants harvested after 12 months (0.69%) and 6 months (0.41%) after planting. Sobte *et al*. (1951) reported that the yield of roots and alkaloid content increases with the age of the plant. Our findings are similar with the findings of earlier workers. Previously, it was reported that *R. serpentina* roots contained not less than 1% total alkaloids (WHO, 1996). In our study *R. serpentina* roots contain alkaloid more than 1 percent. Different harvesting stages/time influenced the root yield and alkaloid content of *R.serpentina* (Chatterjee, *et al*. 1956). Maximum alkaloids content (1.97%) was found in the roots harvested after maturity i.e. in the month of December after 18 months of planting. While minimum alkaloids content was 0.35% in the roots of crop harvested after 6 months of planting. Study revealed that transplantation of seedlings raised through seeds is better method of cultivation of *R. serpentina*. It can be concluded that highest root yield and alkaloid content can be obtained from the crop raised though seeds harvested after 18 months of planting in the month of December.

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