REGENERATION OF *ATROPA ACUMINATA* ROYLE AT KUZA-GALI FOREST ENCLOSURE OF GALIS FOREST DIVISION

Shakeel Haider Zaidi, Assistant Economic Botanist, ***Anwar Ahmad Khan,*** Director, Biological Sciences Research Division and ***Muhammad Muslim,*** Research Assistant, Pakistan Forest Institute, Peshawar.

ABSTRACT

Atropa acuminata Royle plant is well-known for its active constituents extracted from leaves and roots. It is widely used in a number of pharmaceutical preparations. In order to develop regeneration techniques of the plant for its conservation experiments on regeneration and cultural studies were conducted for 3 consecutive years in its natural habitat. Standardized techniques of raising seedlings from seeds or by root-stock were developed. The plant was successfully regenerated in its natural habitat. Suitable spacing for planting and appropriate doses of NPK fertilizer required for better growth and yield was determined. The plant is perennial at higher hills and good yield of leaves was recorded during second and third year's growth of the plant. Roots of *A. acuminata* attained exploitable size after three years growth.

INTRODUCTION

Atropa acuminata (Indian Belladonna) is one of the most important pharmacopoeial plant reputed for its mydriatic and sedative effects since long in western countries. The commercial drug is obtained from leaves, flowering tops and roots of the plant. The herb (leaves and flowering tops) is particularly used in a number of pharmaceutical formulations either in the form of crude extracts such as tincture, powder, ointments or pure alkaloids etc., while roots are generally used in preparations such as liniments, plasters and suppositories (Anon 1982).

The alkaloid concentration as reported by various workers ranged from 0.13 to 0.73% (Denston, 1945, Malik and Imam 1968; Hussain 1977). The main alkaloids are atropine, hyoscyamine alongwith its racemic forms and traces of scopolamine (hyoscine). These alkaloids act as antispasmodic, used in the treatment of renal, bilary colic and gripping pain during stomach disorder. They also posses anticholinergic properties and check the action of excretory glands and relax smooth muscle tissue etc. The plant also gained importance in ophthalmology due to dilatory effect of atropine on pupil of eyes which has been used as a basic constituent in numerous proprietary medicines.

A. acuminata is a mesophytic, tall perennial, bushy herb, 0.70 to 1.5 m in height with dichotomously branched system. It grows wild in moist and shady localities as under-growth in mixed coniferous forests of north-western Himalayan mountain ranges at an altitude ranging from 2800 to 3000 m especially at Galiat, Hazara, Swat, Dir and Chitral (Nasir and Ali 1972). The plants are uprooted during collections, its large scale and indiscriminate collection by the contractors for longer periods in the past has resulted in its extinction from more accessible forest areas. Presently only stray plants in small patches are sparsely distributed in far flung inaccessible hilly areas thus making exploitation uneconomical.

Keeping in view the importance of the plant in pharmaceutical industry and to conserve germplasm resources from annihilation, effort was

made to develop regeneration and propagation techniques of this endemic plant in the hill forests at Kuza-gali which is in moist temperate zone at 2500 m. elevation with 1650 mm annual rainfall-the plant natural habitat. Experiments conducted to determine the cultural, agronomical and fertilizer requirements of the plant facilitated the development of package of agro-technology for pilot scale regeneration of the plant in the forest enclosure. Results of long-term studies are presented in this article.

MATERIALS AND METHODS.

Berries of *A. acuminata* collected from Sharan (Kaghan) during 1985 were dried in shade and seeds were sown in petri dishes.

The viability of seed as tested in laboratory was low and irregular (30 to 40%). Pre-treatment of seed with 200 ppm Gibberellic acid for 30 minutes improved the germination by 93 percent (Khan and Zaidi, 1989). Seed were treated with 200 ppm Gibberellic acid for 30 minutes and later sown in 4000 polythene tubes (size 5 x 12 cm) containing a mixture of fine sand, manure and soil (33% each) in November, 1985 in nursery at Peshawar. Watering was provided twice a day (i.e. morning and evening). Seeds started germination after 22 days and it took 70 days for complete germination. The seedlings after maintenance for three months in nursery were transported in April, 1986 to Kuza-Gali. The soil of the area is rich in humus and fairly deep on gentle to moderate slopes. Prior to laying out trials, 24 soil samples from 0-15 cm depth and 24 soil samples from 15-30 cm depth were detained from the experimental area and analyzed for physico-chemical characteristic which indicated a sandy loam soil with a pH value of 7.5, organic matter 3.61% and total initial Nitrogen as 0.18%, Phosphorus measured as P_2 05 was 26 ppm and exchangeable Potassium as K_20 was 291 ppm. The experiment was set up in a split plot design replicated four times. The size of sub plot was 12 m^2. The variable to be tested were split application of artificial fertilizer (NPK) in two different combinations i.e. (i) N 150 + P 60 + K 30 kg/ha (ii) N 200 + P 90 + K 60 kg/ha and effect of various spacings on the growth and yield of leaves and roots of the plant. The seedlings were planted in well prepared replicated plots at four spacings viz; 45 x 30cm, 45 x 45 cm, 60 x 45 cm and 60 x 60 cm as per experimental design in April, 1986, thus covering a total area of 438 m^2 on south-western aspect with moderate slope. The predominant tree species were *Pinus willichiana* and *Abies pindrow* which formed mixed canopy on that aspect.

Two weeding and hoeings were carried out during the month of June and August each year. First split doses of NPK fertilizer were given after Ist weeding in late June while second split doses were applied as side dressing immediately after second weeding during August according to lay out plan for three consecutive years. Survival percentage of the plant recorded during first year growth in August was 74%, followed by 68% in the second year and 53% during 3rd years growth respectively. Thus average survival percentage through out the period of experiment remained upto 65%. First leaves plucking was carried out four months after planting, while leaves were plucked two times i.e. August and September in second and 3rd year's growth respectively. Yield of fresh leaves under different treatments were recorded in kg/plot. Root was also dug out in November, 1988, after 3 year's growth and weight of fresh root was recorded in kg/plot.

After yield determination, root-stocks were kept in moist pit covered with soil and humus to prevent it from drying. The pit was later reopened in April, 1989 and roots were divided into ½ half 1/3rd and ¼th portions, each having

an average number of one to two buds. The different sized pieces and whole roots were planted in RCB design, replicated four times on 5th May, 1989. Thus 30 root-shoot pieces and whole root-shoots were planted in each plot (6 m^2) at a spacing of 30 x 30 cm. Final observation on the regeneration of root-shoot cuttings were recorded on 31st August 1990, and 1991 respectively at the end of rainy season.

Table 1. Fresh leaves yield (kg/plot)

Spacings (cm)	Fertilizer mixture (NPK)			Pooled mean for spacing
	F1	F2	Mean	
		1986		
45 x 30	0.673	0.830	0.751	-
45 x 45	0.570	0.656	0.613	-
60 x 45	0.578	0.546	0.562	-
60 x 60	0.344	0.463	0.404	-
Mean	0.541	0.624	-	-
		1987		
45 x 30	3.415	3.920	3.668	-
45 x 45	2.940	3.405	3.172	-
60 x 45	2.185	2.666	2.425	-
60 x 60	2.414	2.455	2.434	-
Mean	2.738	3.112	-	-
		1988		
45 x 30	3.745	4.148	3.946	2.788*
45 x 45	4.036	4.280	4.158	2.647
60 x 45	3.293	3.355	3.324	2.103
60 x 60	2.791	3.156	2.973	1.937
Mean	3.466	3.734	-	-

RESULTS AND DISCUSSION

Mean fresh leaves yield of *A. acuminate* in kg/plot (12 m^2) as affected by various spacings and fertilizer treatments is given in Table 1.

Pooled mean for fertilizer	2.248	2.490*	-	

LSD as 5% for fertilizer	1986 NS	1987 0.132	1988 0.155	Pooled 0.068
Plant spacing	0.030	0.058	0.080	0.026

F1 = N150 + P60 + K30 kg/ha
F2 = N200 + P90 + K60 kg/ha

*Mean difference is significant at 5% probability level of significance.

Projection of yield data on hectare basis indicated that estimated yield of fresh leaves increased with the duration of times i.e. from 2nd years to 3rd years growth (2nd year, 2.44 tonnes/ha and 3rd year 3.00 tonnes/ha) as compared to low yield recorded during first year growth (485 kg/ha). The plant is responsive to artificial fertilizer and application of split doses of NPK (N200, P90 and K60 kg/ha gave higher yield of fresh leaves (2.08 tonnes/ha), as compared to lower doses of NPK fertilizer mixture i.e. N150, P60 and K30 kg/ha (1.87 tonnes/ha). These results confirmed the findings of Gulati et al 1976 and Hussain 1977 respectively. It was further observed that plant spaced at 45 x 30 cm gave a significant increase in the yield of fresh leaves (2.32 tonnes/ha) as compared to other spacings viz. 60 x 45 cm and 60 x 60 cm (1.75 and 1.61 tonnes/ha) respectively. No significant difference was observed in the mean leaves yield of 45 x 30 and 45 x 45 cm spacing treatments. Thus 45 x 45 cm spacing was suitable as it utilized 38% less of the planting material and saved labour incurred during planting operations.

Data on fresh root yield harvested at the age of 3 year's growth is tabulated in Table 2.

Table 2. Mean fresh root yield (kg/plot) as affected by fertilizer and various spacing treatments.

Fertilizer treatment	Spacing (cm)				Mean
	45 x 30	45 x 45	60 x 45	60 x 60	
F1	3.38	2.82	2.41	2.26	2.72
F2	3.75	3.15	2.81	2.46	3.04
Spacing mean	3.57	2.98	2.61	2.36	-

Higher roots yield was obtained in plants spaced at 45 x 30 cm spacing and fertilized with split doses of fertilizer mixture (N 200, P 90 and K 60 kg/ha) as compared to lower doses of

fertilizer mixture (F1) and other spacings, which confirmed previous results. The leaves and roots were later dried in shade for seven days and on drying both lost 80% and 30% moisture respectively.

Leaves and root samples were analyzed in Chemistry Branch. These contained 0.45% and 0.45% atropine contents respectively. It compares favourably with the limits as laid down in British Pharmacopoeia (roots 0.4% and leaves 0.3%)

Data on the regeneration of root-shoot cuttings recorded at the end of rainy season for two consecutive year's is tabulated in Table 3.

Table 3. Regeneration of *A. acuminata* from root-shoot cuttings recorded at the end of August,1990 and 1991 respectively

Treatment Size of root-shoot cutting	1990		1991	
	Number of root-shoot cutting regenerated out of 120 planted/Rep	Regeneration %	No. of root-cutting regenerated out of 120 planted/ Rep	Regeneration %
Full	39	33	42	35
½	35	29	41	34
¾	33	28	39	32
¼	38	32	35	29
Mean		30		33

Planting various portion of root-shoot cuttings did not show any significant difference on sprouting percentage. The regeneration percentage of root-shoot cuttings during second years growth was better (33%) as compared to first year regeneration (30%).

It was further observed that whole root-shoot though gave higher number of sprouts as compared to different root-shoot cuttings. Planting of ¼th portion of root-shoot cuttings would be economical and beneficial for raising large number of plants as compared to other root-shoot cuttings.

CONCLUSION

Atropa acuminata constitute an important minor forest produce resources which have dwindled over the years. The plant requires 3-4 years for optimum growth and life cycle completion. The vegetative portion of the plant dries up in winter and a number of vigorous shoots develop from root buds depending upon the age and size of the plant in the spring. Experiments indicated that viability of seed could be improved by pretreatment of seeds with 200 ppm Gibberellic acid for 30 minutes. This treatment also reduced germination periods. Planting could be done either

from seedlings or by root-shoot cuttings. The later method was not practically feasible, as large number of root-stocks (40000 root-shoot cuttings) would be required for one hectare area at 45 x 45 cm spacings. Fresh leaves yield was low during first year growth because fewer number of shoots were produced and it increased with the passage of time in 2nd year's to 3rd year's growth. Root took 3 year's to attain marketable size for harvesting and utilization. The following measures are suggested to prevent degradation of this specie in its natural habitat.

i. 3-4 years' rotation for leaves collection and root extraction of *A. acuminata* plant may be adopted in various forest blocks to provide sufficient time for regeneration and perpetuation of this endemic drug specie.

ii. For artificial regeneration, three months old seedlings can be planted at 45 x 45 cm spacings during the rainy season for re-stocking the area where the population of the plants have depleted. The regeneration percentage of the plant as worked out in various field experiments ranged from 40-66% in the forest enclosure.

iii. Application of NPK fertilizer would be helpful in the early establishment of plants. The regeneration area may be later on closed for 3-4 years to complete regeneration and maturity of the species. This practice would help to conserve germplasm resources and ensure future sustained supply from the hilly areas.

ACKNOWLEDGEMENTS

The authors are grateful to USDA PL-480 authorities for their financial assistance for this project.

REFERENCES

Anonymous 1982 Markets for selected medicinal plants and their derivatives. International Trade Centre UNCTAD/GATT. Geneva pp.83-86.

British Pharmacopoeia 1953 "*Atropa belladonna*". The Pharmaceutical Press 17 - Bloomsburry square London WC1. pp.69-73.

Denston, T.C. 1945 A text book of pharmacognosy. 4th Edition Sir Issac Pitman and Sons Ltd London pp 100-202.

Gulati, B.C., N.A. Qureshi and Taj-ud-din 1976 Cultivation of Belladonna in Kashmir (An Appraisal) Extract from "Cultivation and utilization of medicinal and aromatic plants'" by Atal, C.K and B.M. Kapoor (1977). Reg. Lab. Jammu-Tawi India pp. 46-53.

Hussain, Akhtar (Dr.) 1977 Belladonna and its cultivation in India. Farm bulletin No.5 Central Indian Medicinal Plants Organization National Botanic Gardens Campus Lucknow India pp 1-8.

Khan, A.A. and S.H.Zaidi 1989 Propagation and regeneration technology of pharmacopoeial medicinal plants of temperate regions of Pakistan. Biological Sciences Research Division Bulletin No.8 Pakistan Forest Institute, Peshawar pp. 3-4.

Malik M.N and S.M. Imam 1968 Chemical evaluation of some drug plants of West. Pakistan Pak. Jour. For 18. (4): 511-31.

Nasir, E. and S.I. Ali 1972 Flora of West Pakistan. Fakhri Printing Press Karachi pp-638-39.