Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 1871-1875
Received: 19-07-2020
Accepted: 21-08-2020

Rayees A Bhat
Research Scholars Division of Silviculture and Agroforestry, Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir, India

GM Bhat
Associate Professor, Division of SAF, Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir, India

NA Pala
Division of Silviculture and Agroforestry, Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir, India

FA Shahkhan
High Mountain arid Agriculture Research Institute Stakna, SKUAST-K, Leh, India

Huzaifa Majeed
Research Scholars Division of Silviculture and Agroforestry, Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir, India

Nida Rafiq
Research Scholars Division of Silviculture and Agroforestry, Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir, India

Corresponding Author:
GM Bhat
Associate Professor, Division of SAF, Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir, India

Propagation of *Acorus calmus* through rhizomes and extraction of essential oil from its parts

Rayees A Bhat, GM Bhat, NA Pala, FA Shahkhan, Huzaifa Majeed and Nida Rafiq

Abstract
Acorus calamus is growing naturally in wetlands of Kashmir, an attempt was made to propagate it through rhizomes collected from natural habitat in open nursery conditions. In he present study rhizomes of Acorus after collection from the natural habitat were divided into four different diameter classes viz D_1 (< 0.75 cm), D_2 (0.75to 1.5 cm), D_3(1.5 -2.25) and D_4 (2.25 to 3.0).The rhizomes of these different classes were then sown in well prepared beds at four different spacing's, S1 (60 cm × 30 cm), S2 (60 cm × 45 cm), S3 (60 cm × 60 cm) and S4 (60 × 75 cm).The rhizomes falling in the higher diameter class D_4 (2.25 to 3.0) and at planted at spacing of (60 cm × 75 cm) attained cent percent survival, maximum number of leaves per plant (11.80). The trend was same for other growth parameters *viz*., leaf area (10.53cm^2), leaf dry weight (11.38g/plant), rhizome length (16.75cm), rhizome diameter (5.76mm), above ground biomass (18.50g/plant), below ground biomass (75.50g/plant) and total biomass (94.0g/plant) which was also recorded higher in the higher diameter class of rhizomes D_4 (2.25-3.0 cm) and at higher spacing of S_4(60 cm × 75 cm).Essential oil yield was obtained from leaves and rhizomes of *Acorus calamus* . In leaves maximum essential oil content was obtained from spacing S_4(60cm x 75cm) 0.0115ml/plant and minimum essential oil content was obtained from lower spacing (60cm x 30cm) 0.0067 ml/plant . Similarly, in rhizomes maximum essential oil content was obtained from wider spacing (60cm x 75cm) 0.550ml/plant (16.27kg/ha) and minimum essential oil content was obtained from narrow spacing (60cm x 30cm) 0.212 ml/plant (10.45kg/ha).

Keywords: Acours, diameter, essential oil, rhizomes, spacing

Introduction
The cultivation of medicinal and herbal plants has assumed greater importance in recent years due to their tremendous potential in modern traditional medicine, cosmetic and fragrance industries. One of the earliest records of sweet flag is the *calamus* of the Bible. The first record of sweet flag cultivation was in 1574 A.D. by the Austrian botanist Clusius, who obtained a rhizome from Asia Minor and propagated it Medicinal plants also called as herbs, herbal medicines, pharmacologically active plants or phytomedicinals) remain the dominant form of medicine in most of the countries. *Acorus calamus* is a perennial plant with creeping and extensively branched, aromatic rhizome, cylindrical, up to 2.5 cm thick, purplish- brown to light brown externally and white internally. Sweet flag (*Acorus calamus*) is mentioned in Ayurveda and belongs to the genus *Acorus* L. of the family Acoraceae and is widely distributed temperate to sub temperate regions. It is commonly used in traditional medicinal systems of Asian and European countries to treat appetite loss, diarrhoea, digestive disorders, bronchitis indigestion, chest pain, nervous disorders. *Acorus calamus* Linn. Commonly known as Sweet flag, belongs to the family Acoraceae (Adoraceae). The family Acoraceae comprises about 110 genera and more than 1, 800 species. In Ayurvedic medicine *Acorus calamus* is an important herb and is valued as a "rejuvenator" for the brain and nervous system and as a remedy for digestive disorders. The rhizome of *Acorus calamus* is used for various medicinal purpose mainly appetite, fever, stomach cramps, tooth ache and cholic (Divya *et al.*, 2011) [1]. Propagation through rhizome is used to produce a plant identical in genotype with the mother plant. There are two methods of reproduction sexual and asexual. We get true to type plants when we multiply plants by asexual means. *Acorus calamus* was found to grow at an altitude of 1000-2300 m.

(Tiwari *et al.* 2012) [10] studied the effect of spacing on rhizome yield of *Acorus calamus* L. at collage of agriculture Bilaspur. Harvest of crop after one growing season gave maximum rhizome yield at wider spacing (40 × 40 cm) compared to closer spacing of 20 × 20 cm. The study also illustrates the possibility of optimizing rhizome yield of *Acorus calamus* by manipulation in the time of planting and harvesting as well as maintaining proper planting space. (Sunga *et al.* 2013) [9] reported that *Acorus calamus* has been an item of trade in many

cultures for thousands of years. It has been used medicinally for a wide variety of ailments and its aroma makes calamus essential oil valued in the perfume industry. The essence from the rhizome is used as flavour for pipe tobacco. When eaten in crystallized form, it is called "German ginger". In Europe *Acorus calamus* was often added to wine and the root is also one of the possible ingredients of absinthe. It is also used in bitters. (Rao, 2002) [5] conducted the study on Biomass yield, essential oil yield and essential oil composition of rose-scented geranium (*Pelargonium* species) as influenced by row spacing and intercropping with corn mint (*Mentha arvensis).* The field investigation demonstrated that, 60×30 cm (60 cm between the rows and 30 cm between the plants within the rows) spacing is optimum for rose-scented geranium for harvesting high biomass and essential oil yields and good quality essential oil.

In Kashmir the plant is growing naturally in different Wetlands only, Locally called as Via gander (Kashmiri). It is traditionally used for the treatment of different kinds of diseases. Each part of Plant is used for treatment of different kinds of diseases under traditional and ayurvedic systems. The main ailment categories that are treated include Goiter, gastrointestinal, skin diseases, Renal/urinary disorders, muscular/skeletal disorders, respiratory diseases, eye diseases, fever and others. (Rayees 2018) [6], reported that the livelihood security from *Acorus calamus* depends on multitude of household socioeconomic and biophysical characteristics like education, size of family, size of land holding, herd size, main occupation, family labour, gross annual income, proximity to wetlands, frequency of wetland visit and forest resource possession. The *Acorus* based cottage industry is the 5th major component of household economy and employment in rural Kashmir. Keeping the multifarious uses of acorus in view the present study was undertaken to propagate it asexually by rhizomes and to extract oil from its different Parts by Hydro distillation.

Material and Methods

The present investigation entitled as "Propagation of *Acorus Calmus* through rhizomes and extraction of essential oil from its Parts." was carried out at Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Benhama, Ganderbal during 2018.

Study area

The study was conducted in the central district of Kashmir valley, Ganderbal. The Ganderbal district is located between 34.23°N Longitude and 74.78°E Latitude. There are seven tehsils in the district namely, Ganderbal, Kangan, Lar, Waliwar, Tullmulla, Nagbal and Gutalibagh. and comprises of nine blocks *viz*., Ganderbal, Kangan, Lar, Phaag, Gund, Manigam, Batwina, Wakoora and Shearpathri. The literacy rate of the district is 59.98% (male 68.85% and female 45.71%). The district experiences both temperate and sub-alpine conditions and is well known for excessive annual rainfall (700 mm) and temperature varying from 5 to 20^{o}C**.** The rhizomes were collected from the natural habitat (different wetlands of kashmir) and were divided into four different classes viz D_1 (< 0.75 cm), D_2 (0.75to 1.5 cm), D_3(1.5 -2.25) and D_4 (2.25 to 3.0).The rhizomes of these different classes were then sown in well prepared open nursery beds at four different spacing's, S_1 (60 cm × 30 cm), S_2 (60 cm × 45 cm), S_3 (60 cm × 60 cm) and S_4 (60 × 75 cm) in a replicated manner during January 2018. Following observations were recorded at the end of first growing season

- No. of leaves per plant were counted manually.
- Leaf area (cm^2): It was measured by leaf area meter.
- Leaf dry weight (kg): Leaves were oven dried till constant weight was achieved and dry weight was determined by using top pan balance.
- Rhizome length (cm): The rhizome length was measured in cms by using measuring scale.
- Rhizome diameter (cm): The diameter of rhizome was measured in mm with the help of digital calliper.
- Above-ground biomass (kg ha^{-1}): leaves were harvested and weighed by using top pan balance.
- Below-ground biomass (kg ha^{-1}): Rhizomes were harvested and weighed by using top pan balance.
- Total biomass (kg ha^{-1}): Above ground biomass + below ground biomass

Extraction of oil content of *Acorus calamus* L., by Hydro distillation

In order to extract essential oil by Hydro distillation, all the plots were harvested once in the month of Oct. after calculating the rhizome yield, the rhizome were subjected to Hydro distillation in Clevenger apparatus. Essential oil content present in the rhizomes and leaves were estimated once at the time of harvest. The following observations were recorded at the end of growing season.

Essential oil (ml)/plant

Statistical analysis and interpretation: The data obtained was subjected to statistical analysis using RBD. The vriation between factors and their interaction were tested at 5% level of significance as per the standard statistical procedure.

Results and Discussions

Propagation through rhizome is used to produce a plant identical in genotype with the mother plant. Although *Acorus calamus* grows in wetlands of Kashmir at an altitude of 1000-2300 m, an attempt was made to propagate it through rhizomes collected from natural habitat and made into different diameter classes (04) and different spacing (04) were given and were sown under rain fed conditions at Faculty of Forestry. All the diameter classes of the rhizomes rooted during first growing season, but there was a difference in their growth characteristics. Rhizomes of higher diameter class 2.25-3.0 cm showed better performance under field conditions at a spacing of 60 cm × 75 cm. The reason may be higher diameter class, more robust growth and more spacing, less competition and maximum growth. The rhizomes falling in the higher diameter class (D_4) of 2.25-3.0 cm and at higher spacing of 60 cm × 75cm (S_4) attained maximum number of leaves per plant (11.80). The trend was same for other growth parameters *viz*, leaf area (10.53cm2), leaf dry weight (11.38g/plant), rhizome length (16.75cm), rhizome diameter (5.76mm), above ground biomass (18.50g/plant), below ground biomass (75.50g/plant) and total biomass (94.0g/plant) which were also recorded higher in the higher diameter class of rhizomes 2.25-3.0 cm (D_4) and at higher spacing of 60 cm × 75 cm (Table 1-8). The results were in confirmation with (Tiwari *et al.* 2012) **[10]** who studied the effect of spacing on rhizome yield of *Acorus calamus* L. at collage of agriculture Bilaspur and found that harvest of crop after one growing season gave maximum rhizome yield at wider spacing 40 × 40 cm compared to closer spacing of 20 × 40 cm. The study also illustrates the possibility of optimizing rhizome yield of *Acorus calamus* by shifting the time of planting season and harvesting time as well as maintaining proper planting space.

(Eltayb *et al*. 2013) [2] also recorded higher values of height, number of leaves, number of branches and survival in wider spacing as compared to narrow spacing's in case of *Morus* species. The high number leaves and branches per plant at the wider planting space compared to those of narrow planting space designates the effect of competition on foliage yield and growth. Our results also got support from the findings of (Sofi 2017) [8], who conducted a study in Faculty of Forestry and reported that propagation of *Arundo donax* through the rhizomes of higher diameter class 40-50 mm raised in open nursery beds were much superior than those grown in polybags in terms of different growth parameters *viz*., height, collar diameter, number of culms/clump, survival, number of nodes, rhizome weight, leaf area, biomass/clump, number of rhizomes/clump, fresh weight/clump, dry weight/clump and moisture content. Our findings were also in confirmation with (Nida 2018) [4] who conducted a study at Faculty of Forestry to determine the effect of spacing on growth and yield of *Bergenia cilita* L. and found that among the four spacing's (60 × 30 cm) (60 × 45 cm) (60 × 60 cm) and (60 × 75 cm), higher spacing (60 × 75 cm) was found to produce more biomass and rhizome yield as compared to other spacing's. It was also found that with increase in spacing, height also increased significantly. Number of leaves and biomass also increased in wider spacing, thereby corroborating our findings that wider spacing can be used to achieve better results for propagation of *Acorus calamus*.

Extraction of oil content of *Acorus calamus* L. by Hydro-distillation

Results from the present study indicated a significant effect of different spacing's on essential oil yield. Essential oil yield was obtained from leaves and rhizomes of *Acorus calamus*. In leaves maximum essential oil content was obtained from wider spacing (60cm x 75cm) 0.0115ml/plant(0.34kg/ha) and minimum essential oil content was obtained from narrow spacing (60cm x 30cm) 0.0067 ml/plant (0.32kg/ha). Similarly, in rhizomes maximum essential oil content was obtained from spacing (60cm x 75cm) 0.550ml/plant (16.27kg/ha) and minimum essential oil content was obtained from narrow spacing (60cm x 30cm) 0.212 ml/plant (10.45kg/ha). The present finding is in agreement with the results of (Sajad 2017) [7], who reported maximum essential oil yield from spacing 40cm x 60cm (24.20 kg/ha) and minimum essential oil yield from spacing 100x 120cm (0.81kg/ha). (Nigussie *et al.* 2015) [3] also studied the effect of plant population density (four intra-rows *viz*., 40, 60, 80, 100 cm and four inter-row plant spacing's of 60, 80, 100, 120 cm) on growth and yield of *Artemisa* (*Artimesia annua* L.) and found that essential oil yield (23.39 kg/ha) was attained due to spacing combination 40 cm intra-row and 60 cm inter-row spacing. Essential oil extracted from different parts of the plant was directly proportional to the essential oil yield.

Plate 1: Germination of *Acorus calamus* L. Extraction of oil content of *Acorus calamus* L. by Hydro distillation

Table 1: Effect of diameter class, spacing and their interaction effect (diameter classes × spacing) on number of leaves plant^{-1} of *Acorus calamus* L.

Diameter classes	Spacing				Mean
	S_1	S_2	S_3	S_4	
D_1	4.50	5.75	7.25	8.25	6.43
D_2	5.25	6.25	8.50	9.75	7.43
D_3	7.25	9.25	9.80	10.80	9.27
D_4	8.40	9.50	10.75	11.80	10.11
Mean	6.35	7.68	9.07	10.15	
Treatment	CD 5%	Treatment	CD 5%	Treatment	CD 5%
D	1.15	S	1.13	D × S	1.15

D_1: <0.75 cm, D_3: 1.5-2.25 cm, S_1: 60 cm × 30 cm, S_3: 60 cm × 60 cm
D_2: 0.75-1.5 cm, D_4: 2.25-3.0 cm, S_2: 60 cm × 45 cm, S_4: 60 cm × 75 cm

Table 2: Effect of diameter classes, spacing and their interaction effect (Diameter classes × spacing) on leaf area (cm^{-2}) of *Acorus calamus* L.

Diameter classes	Spacing				Mean
	S_1	S_2	S_3	S_4	
D_1	4.25	5.30	6.25	6.50	5.57
D_2	7.01	7.23	7.33	8.83	7.60
D_3	8.17	9.43	9.73	9.77	9.27
D_4	9.83	10.03	10.23	10.53	10.15
Mean	7.31	7.99	8.38	8.90	
Treatment	CD 5%	Treatment	CD 5%	Treatment	CD 5%
D	NS	S	1,42	D X S	1,41

Table 3: Effect of diameter class, spacing and their interaction effect (Diameter classes × spacing) on rhizome diameter (mm) of *Acorus calamus* L.

Diameter classes	Spacing				Mean
	S_1	S_2	S_3	S_4	
D_1	2.26	2.51	3.39	4.01	3.04
D_2	3.01	4.11	4.49	4.61	4.05
D_3	4.01	4.99	5.01	5.51	4.88
D_4	4.49	5.09	5.26	5.76	5.15
Mean	3.44	4.17	4.53	4.97	
Treatment	CD 5%	Treatment	CD 5%	Treatment	CD 5%
D	NS	S	0.68	D x S	0.68

Table 4: Effect of diameter class, spacing and their interaction effect (diameter classes × spacing) on dry weight (g/plant) on leaves of *Acorus calamus* L.

Diameter classes	Spacing				Mean
	S_1	S_2	S_3	S_4	
D_1	3.40	4.25	4.50	6.87	4.70
D_2	5.48	5.88	6.62	7.87	6.46
D_3	8.50	9.50	9.85	10.25	9.50
D_4	9.85	10.50	10.87	11.38	10.65
Mean	6.80	7.53	7.96	9.09	
Treatment	CD 5%	Treatment	CD 5%	Treatment	CD 5%
D	NS	S	!.76	DXS	1.76

Table 5: Effect of diameter class, spacing and their interaction effect (diameter class × spacing) on above-ground biomass (g/plant) [fresh weight of leaves] of *Acorus calamus* L.

Diameter classes	Spacing				Mean
	S_1	S_2	S_3	S_4	
D_1	6.75	9.00	10.96	11.25	9.49
D_2	11.76	12.62	13.75	14.80	13.23
D_3	13.26	15.76	16.25	17.49	15.69
D_4	16.50	17.12	18.25	18.50	17.59
Mean	12.06	13.62	14.80	15.51	
Treatment	CD 5%	Treatment	CD 5%	Treatment	CD 5%
D	NS	S	2.96	D X S	2.96

Table 6: Effect of diameter class, spacing and their interaction effect (diameter class × spacing) on below-ground biomass (g/plant) [rhizome fresh weight] of *Acorus calamus* L.

Diameter classes	Spacing				Mean
	S_1	S_2	S_3	S_4	
D_1	37.15	40.25	42.63	44.70	41.18
D_2	43.12	54.87	62.88	69.12	57.49
D_3	56.87	67.25	71.60	73.25	67.24
D_4	62.88	72.63	73.75	75.50	71.19
Mean	50.00	58.75	62.71	65.64	
Treatment	CD 5%	Treatment	CD 5%	Treatment	CD 5%
D	NS	S	3.86	D X S	3.83

Table 7: Effect of diameter class, spacing and their interaction effect (diameter class × spacing) on total biomass (g/plant) [fresh weight of leaves and fresh weight of rhizome] of *Acorus calamus* L.

Diameter classes	Spacing				Mean
	S_1	S_2	S_3	S_4	
D_1	43.90	49.25	53.59	55.95	50.67
D_2	54.88	67.49	76.63	83.92	70.73
D_3	70.13	83.01	87.85	90.74	82.93
D_4	79.38	89.75	92.00	94.00	88.78
Mean	62.07	72.37	77.51	81.15	
Treatment	CD 5%	Treatment	CD 5%	Treatment	CD 5%
D	NS	S	2.57	D X S	2.57

Table 8: Effect of diameter classes, spacing and their interaction effect (diameter classes × spacing) on rhizome length (cm) of *Acorus calamus* L.

Diameter classes	Spacing				Mean
	S_1	S_2	S_3	S_4	
D_1	9.50	9.85	11.48	13.08	10.97
D_2	11.25	11.50	12.25	14.00	12.24
D_3	12.05	13.25	14.75	15.25	13.82
D_4	13.75	14.00	16.25	16.75	15.18
Mean	11.63	12.15	13.68	14.77	
Treatment	CD 5%	Treatment	CD 5%	Treatment	CD 5%
D	NS	S	NS	D X S	NS

Table 9: Total oil content of *Acorus calamus* L. (ml/plant)

Treatments	Leaves Oil (ml/Plant)	Rhizome Oil (ml/Plant)
S1	0.0067	0.212
S2	0.0085	0.302
S3	0.0098	0.456
S4	0.0115	0.550
CD ($P \leq 0.05$)	0.0015	0.030

References

1. Divya G, Gajalakshmi S, Mythili S, Sathiavelu A. Pharmacological Activities of *Acorus calamus*: A review. Asian Journal Biochemical Pharmacy Resources. 2011; 1:57-64.
2. Eltayb MTA, Warrag EEI, Ahmad AE. Effect of Spacing on Performance of *Morus* species. Journal of Forest Products and Industries. 2013; 2(3):13-23.
3. Nigussie A, Lule B, Gebre A. Effect of Plant population density on growth and yield of Artemisia (*Artemisia annua* L.). International Journal of Advanced Biological and Biomedical Research. 2015; 3(4):384-390.
4. Nida R. Ethno medicinal practices and standardization of propagation techniques of *Bergenia ciliata* L., in Kashmir. M. Sc. thesis submitted to Faculty of Forestry, Sher-e-Kashmir University of Agricultural Science & Technology of Kashmir, Shalimar, 2018.
5. Rao BR. Biomass yield, essential oil yield and essential oil composition of rose-scented geranium (*Pelargonium*

species) as influenced by row spacing's and intercropping with corn mint (*Mentha arvensis* Lf *Piperascens malinv*. ex Holmes). Industrial Crops and Products. 2002; 16(2):133-144.
6. Rayees. Ethno medicinal practices and standardization of propagation techniques of *Acorus calmus* L., in Kashmir. M. Sc. thesis submitted to Faculty of Forestry, Sher-e-Kashmir University of Agricultural Science & Technology of Kashmir, Shalimar, 2018.
7. Sajad A. Standardization of cultivation, harvesting and processing techniques of *Artemisia absinthium* in Kashmir. M. Sc. thesis submitted to Faculty of Forestry, Sher-e-Kashmir University of Agricultural Science & Technology of Kashmir, Shalimar, 2017.
8. Sofi F. Status and propagation of *Arundo donax* under temperate conditions of North-West Himalayas. M. Sc. thesis submitted to Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar, 2017.
9. Sunga C, Myoung SP, Yu RL, Tae WK, Seon GD. A standardized bamboo leaf extract inhibits monocyte adhesion to endothelial cells by modulating vascular cell adhesionprotein-1. Natural Resource Practical. 2013; 7(1):9-14.
10. Tiwari RKS, Das K, Pandey D, Tiwari RB, Dubey J. Rhizome yield of Sweet Flag (*Acorus calamus* L.) as influenced by planting season, harvest time and spacing. International Journal of Agronomy. 2012; 10(1):101-108