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Standardization of conventional propagation techniques for four medicinal species of genus *Ficus* Linn.

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The four *Ficus* species, viz. *Ficus racemosa* Linn. (Cluster fig), *Ficus microcarpa* Linn.f. (Chinese or Malayan banyan), *Ficus religiosa* Linn. (Peepal tree) and *Ficus benghalensis* Linn. (Banyan tree) belonging to the '*Nalpamara'* group of medicinal trees possess immense medicinal values. The barks of these four *Ficus* species make a unique formulation to cure various uterine problems in women. Germination percentages of seeds of *F. racemosa*, *F. microcarpa, F. religiosa* and *F. benghalensis* are reported to be 5.0, 2.3, 27.7 and 82.0%, respectively. Therefore, studies were undertaken to standardise and compare various propagation methods for these species. Various seed treatments showed improvement in germination percentage. In *F. racemosa* and *F. microcarpa* there was drastic decrease in seed viability after six months. In *F. religiosa* viability started declining only after 12 months whereas *F. benghalensis* seeds retained satisfactory germination even after 18 months. Studies on vegetative propagation showed that all the four species showed poor rooting of stem cuttings. The percentage success of rooting was 10-12% in *F. racemosa* and *F. religiosa* and 18-20% in *F. microcarpa* and *F. benghalensis*. Air layering was found very successful in all the four species and the percentage success achieved was 80-90%. In *F. racemosa* success rate during May-June was only 40% whereas it was 90% during August–September.

Keywords: Medicinal plants, *Ficus* spp., Seed germination, Seed viability, Stem cuttings, Air layering, Propagation.

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Introduction

The genus *Ficus*, commonly known as Figs, belongs to the family Moraceae and constitutes an important group of trees with immense medicinal value. There are over 600 species of *Ficus*, of which the four species, viz. *Ficus racemosa* Linn. (Cluster fig), *Ficus microcarpa* Linn. f. (Chinese or Malayan banyan), *Ficus religiosa* Linn. (Peepal tree or Sacred fig) and *Ficus benghalensis* Linn. (Banyan tree) are the medicinally important ones. The group of these four species is known as 'Nalpamara' meaning the four trees with milky latex. The barks of these trees together form an important ingredient in many Ayurvedic formulations, used in diabetes, diarrhoea, leucorrhoea, menorrhagia, nervous disorders and vaginal diseases[1]. Roots of *F. racemosa* are useful in treating dysentery and its bark is useful as a wash for wounds, highly efficacious in threatened abortions and recommended in uropathy. Bark of *F. microcarpa* is used in wounds, ulcers, bruises, diarrhoea, dysentery, diabetes and leucorrhoea. Its aerial roots are used to treat dental caries. Fruits of *F. religiosa* are laxative and digestive and bark of this tree is used in the treatment of gonorrhoea, diarrhoea, dysentery and haemorrhoids. Bark of *F. benghalensis* is antidermatic, anti-inflammatory and ophthalmic[1-3].

All these species are distributed all over India. *F. religiosa* requires deep, alluvial sandy loam with good drainage. It grows on a variety of soils including shallow and stony sites or even in rocky cervices. It also thrives on saline soil. The four *Ficus spp*. comes under the priority list of plants to be cultivated and the annual demand of crude drug of these species in the country is estimated to be 200-500 tonnes per annum[4]. Since barks are the useful part in these species, collection of the officinal part often leads to complete destruction of the plant which imposes serious threat on their population. Hence, attempts are to be made for large scale planting of the species in public places, uncultivated areas and forest lands.

The propagation of these four *Ficus spp*. is mainly through seed and vegetative means. Seeds of *F. religiosa* require sunlight to germinate; also constant humidity of the substrate is essential[5].

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Potential of germination of *F. microcarpa* seeds ingested by Rufous-bellied Thrush (*Turdus rufiventris*) has been studied and faster germination rate was observed after the seeds passed through the bird's digestive system[6]. *T. rufiventris* is considered an important disperser of *Ficus* in the urban environment. Early germination of seeds in the presence of *Trichoderma harzianum* has also been reported[7]. Seed germination of *F. racemosa* and *F. hispida* were reported to be unaffected by ingestion by frugivorous bat species[8].

Vegetative propagation through stem cuttings is the main method of propagation in many *Ficus spp*. Softwood cuttings treated with MBC (Carbendazim) solution for 5s, then with IBA at 1000-4000 mg/lt gave 85-100% rooting in many fig trees[9]. Maximum rooting and sprouting of *Ficus glomerata* occurred with 1.0 mg/ml IBA, followed by 0.5 mg/ml IBA. Exogenous application of auxin substantially increased rooting percentage of *Ficus auriculata* and *Ficus glomerata* with the higher concentration of IBA (500 ppm) promoting the best rooting[10,11]. Rooting of branch cuttings of the two sacred *Ficus* tree species, viz. *F. benghalensis* and *F. religiosa* is reported to be poor[12].

The earlier reports lack adequate information on seed germination, seed viability, growth of germinated seedlings and success percentage of vegetative propagation using stem cuttings and air layering in the four *Ficus spp*. of 'Nalpamara' group. Hence the present study was undertaken to standardize and compare various propagation methods in these species which are prerequisites for undertaking any cultivation and conservation program.

Materials and Methods

The present investigation was carried out during 2005–2008 at Aromatic and Medicinal Plants Research Station, Odakkali (Kerala Agricultural University). The station lies between 10°5'40" and 10°6'0"N latitude and 76°32'35" and 76°32'55"E longitude and is located at an elevation of 60 m above MSL.

Propagation by seeds

Seeds of the four species were collected from 12-20 year old plants. The trees for collection of seeds were identified from Ernakulam and Idukki districts of Kerala. The ripened fruits were collected and seeds extracted, washed, dried in shade and stored in air tight containers. Observations on fruit and seed characters were taken and mean values were recorded.

Seed germination studies

Petri plate method was used in this study using completely randomized block design. Separate lots of seeds of the four species were subjected to various treatments. From each lot, 100 seeds each were carefully counted out and kept in petri plates for germination tests. The treatments were: control, soaking in water at 65º C for 10 min., heaping and covering seeds with moist gunny cloth for a week, mechanical scarification by rubbing with sand paper, soaking seeds in 40% sulphuric acid for 10 min., soaking seeds in 10% sulphuric acid for 10 min., soaking seeds in 40% nitric acid for 10 min., soaking in 100 ppm gibberellic acid for 24 h, soaking in dilute solution of acetic acid (pH adjusted to 3.3) for 2 h, soaking in dilute solution of ammonia (pH adjusted to 8.6) for 12 h, soaking in hot water for 10 minutes followed by soaking in gibberellic acid 100 ppm for 24 h. The treatments were replicated thrice. Number of seeds germinated in each species under different treatments was recorded and the percentage of germination worked out. Also the days for starting and completion of germination of each species was also recorded.

Seed viability

Viability of seeds i.e. period up to which seeds show satisfactory germination, was tested under two situations, viz. storing in air tight plastic containers under ordinary conditions and under low temperature condition of 10°C by keeping in refrigerator. 250 g seeds of each species were kept under both the storage situations immediately after collection and processing. Germination percentage was tested at 1, 6, 12 and 18 months after storage in the case of storage under ordinary conditions whereas the seeds under refrigerated conditions were tested at 12 and 24 months after storage. Petri plate method with three replicates of 100 seeds each in completely randomised block design was used for the germination test. The extent of variation in germination percentage from the original level was used as a test of change in seed viability.

Growth of seedlings

In order to assess the growth rate of seedlings of the four *Ficus spp*., seeds were sown in sand beds and immediately after germination and formation of leaves; they were transferred to poly-bags filled with potting medium at one seedling per bag for recording the growth parameters. The design used was randomised block design with five replications using 20 seedlings per treatment. Growth of the seedlings was assessed by recording plant height

and leaf number of six seedlings each at 7, 9 and 11 months after planting.

Vegetative propagation

By stem cuttings

Effect of rooting hormone and thickness of cutting: Stem cuttings of thickness ranging from 5-30 mm diam of the four species were collected and they were clustered into four groups based on diameter of cuttings, viz. 5-10 mm, 10-15 mm, 15-20 mm and 20-25 mm. Under each group, one set was treated with rooting hormone by dipping the cuttings in 1000 ppm IBA for 15-20 minutes, whereas the other set was kept untreated. The statistical procedure used was factorial randomised block design with three replications. Under each treatment, 20 Nos of cuttings of length 30 cm were taken and planted one each in polybags filled with potting mixture to study the influence of stem thickness and rooting hormone on sprouting. The percentage of cuttings which sprouted and got established under each treatment was recorded.

By Air layering

Air layering was done in all the four species during May-June with the start of monsoon. Trees available in the Medicinal Trees Conservation Park of the research station were used in the study. Layering was done in current season's young branches as well as in 1-2 year old branches. In one set of layers no hormonal application was done whereas in another set, the coir pith medium used in layering was soaked in rooting hormone IBA at 1000 ppm. Under each treatment combination, air layering was done in 10 twigs. The experimental design was factorial randomised block design with five replications per treatment. After one and a half months, a slanting cut to half the thickness of stem was given at the base of the layers. The layers were ready for separation in another 2-3 weeks when thick root growth was visible through the transparent plastic cover. The twigs were then removed from the trees and planted in polybags filled with potting medium and the percentage of successful layers and the extent of survival under each treatment were recorded.

In *F. racemosa*, leaf shedding occurred in the plant during July-August period, hence air layered twigs showed poor rooting and survival. The process was hence repeated during August–September when the plants putforth new flushes.

Statistical analyses of the data were carried out and critical difference values at 5% probability level were used for comparison of means[13].

Results and Discussion

Seed propagation

Fruit and seed characters: Observations on fruit and seed characters of the four *Ficus spp.* were recorded and they are presented in Table 1 and Plate 1. The seeds are orthodox in nature. *F. racemosa* has the largest fruit among the four 'Nalpamara' trees. Fruits of this plant are borne in clusters on main trunk and branches whereas in other three species they are borne only on branches. Another distinct difference noticed is that fruit bearing occurs twice a year in *F. racemosa* whereas in the other three species, fruits are formed only once during December to February. Fruits of *F. microcarpa* are very small containing numerous small seeds. Regular fruit setting was not seen in most trees of *F. religiosa* and *F. benghalensis*. In *F. benghalensis* fruits were of two colours, viz. reddish-yellow and bright red. Reddish yellow fruits were slightly larger in size compared to bright red coloured ones.

All the four species of *Ficus spp*. are monoecious; separate male and female flowers are borne on the same plant. Ovary is one celled. The seeds are orthodox in nature. *Ficus* produces a unique fruit called syconium or fig, a fleshy hollow structure lined on the inside with hundreds of tiny one seeded

Table 1 — Fruit and seed characters of *Ficus* spp.

Characters	*F. racemosa*	*F. microcarpa*	*F. religiosa*	*F. benghalensis*
Fruit colour	Yellowish red	Yellow	Pinkish red	Reddish yellow/bright red
Fruit diameter (mm)*	20-24	3-5	4-7	11-17
Fresh weight of single fruit (g)*	11.7	0.35	0.44	2.85
No. of seeds/fruit**	1628	168	118	586
Seed colour	Reddish brown	Sandal	Yellow with red colour at the tip	Light brown
1000 seed weight (g)**	0.9	0.6	1.0	0.68
Fruit ripening season	June-July and December-January	December-January	February–March	January–February

*Mean of ten values; ** Mean of three values

carpels. The fruit and reproduction systems of species in the genus *Ficus* are unique. Each species of *Ficus* has an associated species of agaonid wasp (Hymenoptera: Agaoninae) for pollination. *Ficus* species can only be pollinated by their associated agaonid wasps and in turn, the wasps can only lay eggs within their associated *Ficus* fruit[3,18].

Seed germination: Germination percentages recorded under different treatments are presented in Table 2. Germination percentages in untreated seeds of

Plate 1— Mature trees, leaves, fruits and seeds of *Ficus* spp.: a. *F. racemosa*; b. *F. microcarpa*; c. *F. religiosa*; d. *F. benghalensis*

Table 2 — Effect of seed treatment on seed germination of *Ficus* spp.

Treatment	Germination percentage			
	F. racemosa	*F. microcarpa*	*F. religiosa*	*F. benghalensis*
Control	5.0	2.3	27.7	82.0
Soaking in water at 65º C for 10 minutes	11.0	2.7	52.3	85.7
Heaping and covering seeds with moist gunny cloth for a week	4.0	0.0	26.3	67.7
Mechanical scarification	5.3	2.0	25.7	77.3
Soaking seeds in 40% sulphuric acid for 10 min	1.7	1.1	14.3	86.0
Soaking seeds in 10% sulphuric acid for 10 min	2.3	2.3	46.0	79.3
Soaking seeds in 40% nitric acid for 10 min.	0.0	0.0	0.0	0.0
Soaking in 100 ppm gibberellic acid for 24 h	11.0	2.7	37.7	80.0
Soaking in dilute solution of acetic acid (pH adjusted to 3.3) for 2 h	1.7	0.0	33.7	87.3
Soaking in dilute solution of ammonia (pH adjusted to 8.6) for 12 h	10.7	0.0	48.7	90.0
Soaking in hot water for 10 minutes followed by soaking in gibberellic acid 100 ppm for 24 h	12.0	0.0	44.0	87.3
CD (*P*=0.05)	5.4	NS	17.9	7.4

CD at 5% probability; NS–Non-significant

F. racemosa, *F. microcarpa, F. religiosa* and *F. benghalensis* were 5.0, 2.3, 27.7 and 82.0, respectively. Soaking seeds in hot water at 65ºC for 10 minutes recorded 11.0% germination in *F. racemosa*. Water soaking is beneficial to render the seed coat permeable[14]. Soaking in 100 ppm gibberellic acid for 24 h, soaking in dilute solution of ammonia (pH adjusted to 8.6) for 12-15 h as well as soaking in hot water for 10 minutes followed by soaking in gibberellic acid 100 ppm for 24 h also recorded germination percentage significantly superior to untreated control. This corroborates with earlier finding that gibberellic acid, a growth hormone for promoting seed germination, is considered responsible for mobilization of nutrients[15]. Influence of ammonia in stimulating seed germination has been reported earlier[16]. In *F. microcarpa,* germination capacity of seeds was very poor. The various treatments tested were not effective in improving germination percentage. Some of the earlier reports also show low germination percentage in *F. microcarpa* seeds and this is attributed to the low abundances of the species in the natural forest[17]. For successful pollination and reproduction of *Ficus* species to occur, its associated pollinator wasp must be present[18]. A detailed analysis on seed biology and pollination is required to find out the cause for low seed germination of *F. microcarpa* observed in this study.

Data on *F. religiosa* showed that on an average 30-50% seeds germinated. Untreated seeds showed lower percentage of 27.7% whereas hot water treatment, soaking in 10% sulphuric acid and soaking in dilute solution of ammonia gave significantly higher germination. Seeds of *F. benghalensis* showed higher germination percentage as compared to the other three species. Untreated seeds itself gave good germination of 82%. Soaking in dilute solution of ammonia enhanced the germination to 90%.

In *F. religiosa* germination started on 8th day of incubation, but it prolonged up to 35 days whereas in the other three species, germination completed in 10-15 days.

Seed viability: The data on germination percentage of seeds kept for studying viability of the four *Ficus spp*. are shown in Table 3. In *F. racemosa*, germination percentage decreased drastically on storage. But the seeds kept in refrigerator retained germination of 12.3% even after 12 months. Seeds of *F. microcarpa* failed to germinate after six months. In *F. religiosa*, there was no appreciable reduction in germination percentage during the first year; however by 18th month germination decreased drastically. At the same time, seeds kept in refrigerator did not exhibit appreciable reduction in germination even after 24 months. Refrigerated storage as a method of extending seed viability has been reported in many

studies[19,20]. Germination percentage of *F. benghalensis* did not show appreciable reduction even at 18 months of storage. Seeds stored in refrigerator retained comparable germination at 12th month of observation; but a significant reduction from 89% to 79% was noticed at 24 months of storage.

Growth of seedlings: Data on plant height and leaf number of polybagged seedlings of the four *Ficus spp*. are presented in Table 4. Seedlings of *F. racemosa* showed a fast growth rate compared to the other three species and by 7-8 months the seedlings were ready for transplanting. In *F. religiosa* though initial growth was slow, after 7th month it showed higher growth rate and it attained sufficient size for field planting by 11th month. Growth of seedling was very sluggish in *F. microcarpa* and *F. benghalensis*.

Vegetative propagation

Stem cuttings

Effect of rooting hormone and thickness of cutting: Data on percentage of rooting of stem cuttings are presented in Table 5 and Plate 2. All the four *Ficus spp*. showed poor rooting of stem cuttings. Rooting percentage varied with thickness of cuttings. Higher rooting was noticed in 15-25 mm sized cuttings. Among the four *Ficus spp*., *F. microcarpa* and *F. benghalensis* showed better rooting. In *F. racemosa* and *F. religiosa*, root formation and survival was obtained only in 10-12% cuttings whereas in *F. microcarpa* and *F. benghalensis*, 18-20% survival was obtained. Poor rooting of branch cuttings in *F. benghalensis* and *F. religiosa* has been reported earlier[12]. Dipping of stem cuttings in 1000 ppm IBA did not improve sprouting percentage in all the four species.

Air layering

Data on percentage rooting and percentage of layers successfully established in polybags are given in Table 6 and 7 and Plate 3. Air layering during May –June was successful in *F. religiosa*, *F. benghalensis* and *F. microcarpa* whereas layering in August–September proved successful in *F. racemosa*.

In *F. racemosa* percentage of rooting during May–June was only 40% in old branches and 13-20% in young branches. Leaf shedding was noticed in the plant during the period of root development in the layered twigs which attributes to the low production of successful layers. During August–September, new leaves were formed in the plant and there was 80-90% success in layering. The results indicated that in *F. racemosa* layering is more successful during August-September compared to May-June period.

In *F. microcarpa*, the rooting of old branches was 60-80%, while young branches showed a lower

Table 3 — Viability of seeds of *Ficus* spp.

Particulars	Germination percentage			
	F. racemosa	*F. microcarpa*	*F. religiosa*	*F. benghalensis*
Months after storing				
	a. Stored in air tight plastic jar at room temperature			
1	10.8	2.4	54.3	88.0
6	5.3	0.7	56.3	85.7
12	2.3	0.0	52.3	88.7
18	2.0	0.0	14.7	83.0
	b. Stored in air tight plastic jar and kept in refrigerator			
12	12.3	0.0	59.0	89.0
24	10.0	0.0	52.3	79.0
CD (*P*=0.05)	2.6	0.8	8.3	5.3

Table 4 — Growth of seedlings of *Ficus* spp. in polybags

Name of species	Plant height (cm)			Number of leaves		
	7 MAP*	9 MAP	11MAP	7 MAP	9 MAP	11 MAP
F. racemosa	35.8	67.3	102.7	17.0	54.3	69.0
F. microcarpa	7.8	14.3	18.7	8.3	13.7	11.3
F. religiosa	18.7	33.5	50.0	12.0	19.3	22.7
F. benghalensis	4.5	8.7	8.3	4.7	4.7	4.0
CD (*P*=0.05)	12.3	15.3	18.3	5.8	7.6	9.2

*MAP – Months After Planting

Table 5— Effect of thickness and rooting hormone on rooting of stem cuttings in *Ficus* spp.

Treatments	% Rooting			
	F. racemosa	*F. microcarpa*	*F. religiosa*	*F. benghalensis*
Thickness of cutting (mm)				
5-10	0.0	1.0	1.3	5.3
10-15	4.8	12.3	6.0	11.2
15-20	10.3	19.5	9.9	20.3
20-25	11.5	18.1	10.5	19.5
CD (*P*=0.05)	2.8	3.1	3.2	2.7
Rooting hormone				
Untreated	6.3	13.1	8.6	15.0
IBA 1000 ppm	7.0	12.3	6.7	13.1
CD (*P*=0.05)	NS	NS	NS	NS

NS- Non-significant

Table 6 — Rooting and survival percentage of air layers in *F. racemosa*

Treatments	May-June		August-September	
	% Rooting	% Survival	% Rooting	% Survival
Old branches, untreated	40	36	90	80
Old branches, IBA treated (1000 ppm)	40	40	90	80
Young branches, untreated	13	13	80	70
Young branches, IBA treated (1000 ppm)	20	13	75	70
CD (*P*=0.05)	8.5	10.2	9.0	7.5

Plate 2 — Rooted stem cuttings in *Ficus* spp.: a. *F. racemosa*; b. *F. microcarpa*; c. *F. religiosa*; d. *F. benghalensis*

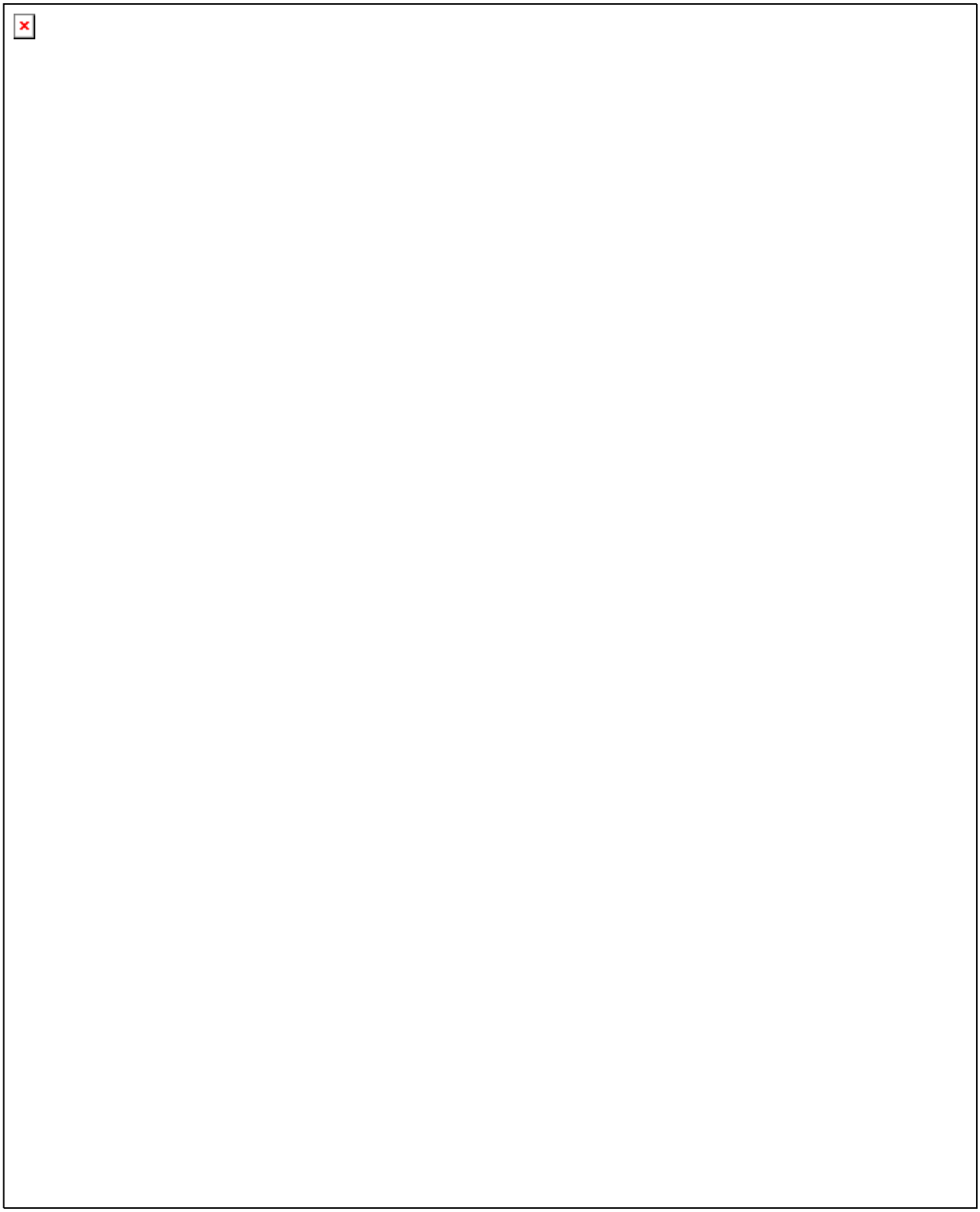

Plate 3 — Air layered twigs of *Ficus* spp.: a. *F. racemosa*; b. *F. microcarpa*; c. *F. religiosa*; d. *F. benghalensis*

percentage of 53-73%. About 50-60% of the layers from old branches survived whereas it was lower (27-33%) in case of young branches.

In *F. religiosa* 80-90% of the layered twigs showed rooting. Rooting was observed in both types of cuttings; however, survival rate was significantly

Table 7 — Rooting percentage and establishment of air layers in *F. microcarpa*, *F. religiosa* and *F. benghalensis*

Treatments	*F. microcarpa*		*F. religiosa*		*F. benghalensis*	
	% Rooting	% Survival	% Rooting	% Survival	% Rooting	% Survival
Old branches, untreated	80	60	90	56	90	80
Old branches, IBA treated	73	50	84	60	90	80
Young branches, untreated	53	27	87	33	90	50
Young branches, IBA treated	60	33	83	40	90	60
CD (*P*=0.05)	6.4	9.2	6.8	7.9	NS	11.2

NS–Non significant

higher in old branches. In *F. benghalensis*, 90% of layers rooted and out of this 50-80% got established. Layers from old branches showed better survival.

Conclusion

The study helped to standardize various conventional propagation techniques and gather information on percentage success of various methods in the four *Ficus spp*. Air layering was found successful in all the four species. Though use of stem cuttings is a speedy and simple method, percentage rooting is very low. In *F. racemosa* and *F. religiosa*, seed propagation can be easily adopted since their seedlings become ready for planting in 8-10 months. Detailed studies are required to find out the cause of poor seed germination in *F. microcarpa* observed in the study.

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