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Variability Studies on Seed Parameters, Oil and Azadirachtin Content of Neem (*Azadirachta indica* A.Juss.) in Tamil Nadu and Karnataka

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A B S T R A C T

Thirty four Plus Trees (PTs) of *Azadirachta indica* were screened based on the tree morphology and biochemical traits to identify the suitable source for high oil and azadirachtin contents in order to establish large scale industrial plantations. Significant was observed among 34 PTs for seed and biochemical traits. Maximum 100-seed weight (30.00g) and 100 kernel weight (12.00g) were recorded in NPT 7, while NPT 34 showed maximum oil content (42.81%) and maximum azadirachtin content was documented in NPT 11 (0.972%) followed by NPT 17 (0.970%). High heritability (broad sense) and genetic gain were observed for all seed and biochemical traits respectively. Seed weight and kernel weight showed significant correlation with azadirachtin content. Among thirty four PTs, NPT 11, NPT 22, NPT 17, NPT 4 and NPT 29 were found superior in terms of oil and azadirachtin contents. Hence these seed and biochemical parameters could be considered as selection criteria for early and positive exploitation of high oil and azadirachtin yielding genotypes. Study confirmed that the existence of substantial genetic variation which can be utilized for genetic resource conservation in gene bank and further tree improvement programmes.

Introduction

Azadirachta indica A. Juss commonly known as neem belongs to family Meliaceae and is one of the important fast growing multipurpose tree species of Indian sub-continent since antiquity. The tree is widely adapted to various climate and soil types. It is commonly found in South Asia and parts of Africa. In India, neem tree occurs in tropical dry deciduous and thorny forests up to an altitude of 1500m. It is a fast growing, hardy, evergreen tree but under extreme conditions, such as extended dry periods, leaf may shed (Tiwari, 1992). It tolerates high temperatures, low rainfall, long spells of drought and salinity. Neem is propagated mainly through seeds. Four to six months old seedlings are ready to plant in the field. Fruiting begins in 4-5 years. In India neem flowers from March to May and fruits mature from June to August.

Seed viability generally ranges from 2-3 weeks after collection, the presence of unsaturated free fatty acids (Oleic acid, 51.3%) in neem oil could be responsible for the quick loss of viability (Chaney and Knudson, 1988). Whether neem is a genuine recalcitrant or short lived orthodox species, however is still nebulous. On the basis of low moisture content of seeds (12.5%), it has been argued that neem is not a recalcitrant species. Again since neem occurs in dry tropical forests, while most recalcitrant tropical species are found in moist tropical forests, it was suggested that neem may have short-lived orthodox seed (Willam, 1985; Devendra, 2015). Oil of neem seeds contain more than 100 active compounds which are together called triterpenoids or limonoids including azadirachtin that would be one of the most important bio pesticides. The kernel contains 40-50% of oil, 18 to 25.4% of crude protein and 0.3 to 1.5% azadirachtin (Diedhiou *et al.,* 2015).

The estimated neem trees present all over India is more than 25 million, of which Uttar Pradesh (55.7%), Tamil Nadu (17.8%) and Karnataka(5.5%) are occupying the first three places respectively. India stands first in neem seed production and about 4, 42,300 tons of seeds are produced annually yielding 88,400 tons of neem oil and 3,53,800 tons of neem cake (Girish *et al.,* 2008).

Wide distribution of neem trees in varying climatic zones confirms its greater adaptability, which most likely is due to broader genetic base. However, very little work has been done to assess this genetic diversity in neem and its utilization for the improvement of existing germplasm for important characters like Azadirachtin content, early bearing and other important features. The effectiveness of tree improvement programme depends upon the nature and magnitude of existing genetic variability and also on the degree of transmission of traits or heritability (Zobel and Talbert 1984), because genetic variation is the fundamental requirement for maintenance and long-term stability of forest ecosystem. The rate of tree improvement can be increased or decreased by influencing the selection differential or heritability, or by reducing the total variance (Bagchi, 1995). The knowledge of genetic variability is considered to provide considerable help in genetic improvement of the species. Hence, the present investigation was envisaged to evaluate the genetic variation in different seed and biochemical parameters collected from various parts of Tamil Nadu and Karnataka.

Materials and Methods

Plus trees of neem

Neem seeds (*Azadirachta indica*) were collected from 125 identified plus trees from various parts of Karnataka and Tamil Nadu during August 2017. The collected seeds were processed as per the standards and moisture, oil and azadirachtin contents were assessed as detailed below. Based on the aza content the top 34 plus trees were identified and they were only used for further analysis.

Moisture content

The moisture content of the seed kernel was determined using ASAE 1998 standard for oil seed. Three samples each weighing 15g was placed in an oven set at 105^0C for 24hours. The samples were then cooled, weighed and the moisture content calculated. Loss in weight is assumed to be moisture loss.

Oil estimation

The oil content was determined by using the standardized Soxhlet method (NF ISO 734-1) that consists in extracting the lipids contained in the matter with hexane for minimum 6 hours. An amount of about 30g of seeds was used. The Soxhlet extractor was equipped at its base with a 250mL flask in which 200 mL of solvent was used.

Estimation of azadirachtin

Azadirachtin content in the neem seed kernel was determined after its extraction, purification and analysis. Azadirachtin content was estimated using standard HPLC method.

Statistical analysis

Observed data was analyzed using SPSS statistical package 'version 2000'.Duncan Multiple Range Test (DMRT) was performed at 5% significance level to observe the homogeneous sub-set between the Plus Trees. Analysis of variance was carried out following the procedure given by Panse and Sukhatme (1976). The variability, heritability in broad sense, genetic advance as percent of mean, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were worked out for seed traits, oil and azadirachtin content as suggested by Lush (1940), Johnson *et al.,* (1955) and Burton (1952).

Results and Discussion

In the present study 34 Plus Trees from Karnataka and Tamil Nadu were screened based on high azadirachtin content (Table 1). Kumaran *et al.,* (1996) collected seeds from 28 one parent families of neem in seven agro climatic zones of Tamil Nadu, India and studied variation and heritability of seed length to seed breadth ratio and 100-seed weight. The significant differences among the plus trees for all the above characters were studied. The physical characterization of neem fruit and seeds revealed that the average mass of 100 fruits was 85.38g, seed weight 20.23g, kernel 8.63g and the moisture content is 57.50% (Table 2). Martın *et al.,*(2010) discussed similar results from various tree born oil seed physical parameters.

There was significant variation among the 34 plus trees for all seed and biochemical characters studied (Table 3). Maximum seed length (16.85 mm) was observed in 29 and minimum (9.54 mm) in NPT 8. Seed diameter showed significant differences among all the plus trees. Three plus trees *viz.,* NPT 15 (7.01mm), NPT 29 (6.89 mm) and NPT 25 (6.88 mm) showed the superiority. 100-seed weight varied from 15.57g (NPT 32) to 30.00 g (NPT 7). NPT 7 (12.90g) recorded maximum 100 kernel weight and followed by NPT 24 (12.00 g). In Husk-Thickness there was no significant difference between plus trees and it ranged from 1.61 mm (NPT 4) to 0.27 mm (NPT 14). Highest decortication percent was recorded by NPT 25 (10.58 %) followed by NPT 16 (10.32 %), NPT 2 (9.63 %) and NPT 27 (9.63 %). The seed moisture content ranged between 39.58 % (NPT 9) and 71.28 % (NPT 32).

The average seed oil content was 41.02% and maximum was recorded in NPT 34 (42.81%). The azadirachtin varied from 0.804% to 0.972% among 34 plus trees; maximum azadirachtin content was documented in NPT 11 (0.972%) followed by NPT 17 (0.970%). Similar result was reported in the one-parent family RJ 32 from Rajasthan with high oil and azadirachtin contents (Kumaran, 1997). Kaushik *et al.,* (2007) revealed the concentration of azadirachtin varied from 200 to 16,000 ppm and azadirachtin content was found to be affected by climate and habitat. Annual variation in azadirachtin content was significant. The highest azadirachtin content was recorded in the neem tree populations growing in the southern part of India.

Table.1 Morphological characters of plus trees from Karnataka and Tamil Nadu

S.No.	Plus Trees	Tree morphology			
		Tree Height (m)	DBH (cm)	Canopy Height (m)	Canopy Width (m)
1.	NPT 1	7.00	62.00	5.30	5.20
2.	NPT 2	9.00	67.50	6.50	8.50
3.	NPT 3	7.50	65.00	5.00	7.00
4.	NPT 4	13.00	127.00	9.00	10.50
5.	NPT 5	14.50	175.00	11.50	13.00
6.	NPT 6	11.00	97.00	9.00	12.00
7.	NPT 7	9.50	104.00	7.50	7.00
8.	NPT 8	9.00	88.00	6.50	6.00
9.	NPT 9	8.50	70.50	6.00	8.50
10.	NPT 10	11.00	104.00	8.50	10.50
11.	NPT 11	12.00	86.50	7.30	7.00
12.	NPT 12	8.30	80.00	5.60	6.00
13.	NPT 13	12.50	115.00	8.00	14.00
14.	NPT 14	10.00	105.00	6.00	8.00
15.	NPT 15	13.00	95.00	7.00	8.00
16.	NPT 16	12.50	95.00	9.80	10.80
17.	NPT 17	9.00	74.00	6.30	7.50
18.	NPT 18	11.50	95.00	8.00	14.00
19.	NPT 19	8.00	78.50	4.00	5.50
20.	NPT 20	10.50	73.00	6.00	5.00
21.	NPT 21	8.00	68.50	4.50	5.00
22.	NPT 22	14.00	112.00	10.50	12.00
23.	NPT 23	8.00	79.50	4.50	8.00
24.	NPT 24	11.00	109.00	8.00	11.00
25.	NPT 25	13.00	94.50	10.00	12.00
26.	NPT 26	7.00	61.00	4.00	5.00
27.	NPT 27	16.00	135.00	13.00	14.50
28.	NPT 28	11.00	98.50	8.00	7.50
29.	NPT 29	9.50	122.50	6.50	5.00
30.	NPT 30	9.50	105.50	7.00	11.00
31.	NPT 31	8.00	83.00	6.00	6.50
32.	NPT 32	8.50	91.50	6.50	10.50
33.	NPT 33	5.50	69.50	3.00	7.50
34.	NPT 34	14.50	136.50	11.50	18.50

Table.2 Physical characteristics of neem fruit and seeds

Fruits	**100 Fruit Weight (g)**	**85.38**
	Length (mm)	16.75
	Width (mm)	11.02
Seeds	Length (mm)	13.72
	Width (mm)	6.08
	100 seed weight	20.23
	100 kernel weight	8.63
	Husk-Thickness (mm)	0.92
	Moisture content %	57.50

Table.3 Mean performance of plus trees for seed and biochemical traits in neem

Plus Trees	Seed Length (mm)	Seed Dia. (mm)	100 seed weight	100 kernel weight	Husk-Thickness (mm)	Decortications %	Moisture content %	Oil Content %	Aza %
NPT 1	11.2	6.14	21.4	8.99	1.11	8.55	52.23	41.23	0.829
NPT 2	13.53	6.61	18.2	7.40	0.98	9.63*	51.79	40.99	0.842
NPT 3	12.89	5.62	17.8	7.90	1.01	7.69	52.04	40.86	0.936*
NPT 4	13.99	6.43	20.20	9.00	1.61	7.09	53.43	39.99	0.943*
NPT 5	14.53	6.37	20.60	8.90	0.69	6.57	59.23	40.14	0.893*
NPT 6	12.65	6.09	21.00	9.50	1.06	9.28	62.56	40.65	0.921*
NPT 7	12.79	5.72	30.00*	12.90*	0.97	7.21	61.35	41.23	0.841
NPT 8	9.54	5.26	22.20	9.10	0.95	7.39	55.8	41.66	0.828
NPT 9	14.85	6.54	22.50	9.80	0.34	8.51	55.92	39.58	0.93
NPT 10	14.49	6.04	13.60	5.30	0.93	8.17	54.08	41.25	0.897*
NPT 11	13.02	5.74	15.80	6.80	1.01	8.44	59.47	40.58	0.972*
NPT 12	14.59	6.75	18.70	7.40	0.62	7.00	53.63	42.22	0.811
NPT 13	12.48	5.52	16.70	6.50	0.66	6.96	52.53	42.01	0.819
NPT 14	12.62	5.56	21.60	9.60	0.27	5.71	63.60*	41.57	0.852
NPT 15	13.33	7.01*	18.70	8.20	1.17	7.37	61.42	41.21	0.845
NPT 16	11.17	6.14	21.30	9.00	0.84	10.32*	64.69*	40.30	0.915*
NPT 17	13.09	6.38	24.20*	10.00*	0.28	8.51	59.92	40.22	0.970*
NPT 18	15.44*	6.44	17.17	7.50	0.56	8.42	65.97*	41.38	0.893*
NPT 19	13.25	5.89	12.74	5.80	0.88	5.10	60.52	42.33	0.838
NPT 20	14.66	6.02	21.33	9.20	1.02	8.22	61.24	40.98	0.931*
NPT 21	15.82*	6.18	23.05	10.20*	1.11	6.57	67.55*	40.82	0.924*
NPT 22	14.93	5.99	17.29	7.80	1.05	9.38*	63.09*	39.99	0.964*
NPT 23	13.57	5.25	18.57	7.50	0.86	5.66	56.65	41.25	0.851
NPT 24	12.68	6.11	26.84*	12.00*	1.34	7.34	58.65	41.56	0.871
NPT 25	15.55*	6.88*	21.69	8.60	1.20	10.58*	52.5	40.29	0.901*
NPT 26	16.02*	6.45	17.86	7.60	0.55	6.56	55.27	41.22	0.874
NPT 27	15.40	6.58	22.95	9.80	1.06	9.63*	56.37	40.76	0.908*
NPT 28	15.64*	6.65*	23.79*	10.20*	1.17	8.12	60.54	40.88	0.894*
NPT 29	16.85*	6.89*	17.80	7.80	1.11	5.31	64.68*	39.86	0.956*
NPT 30	12.38	5.95	20.88	8.60	1.34	6.64	58.55	40.44	0.919*
NPT 31	14.83	5.84	19.35	8.60	0.52	7.23	68.04*	40.56	0.917*
NPT 32	10.89	4.25	15.57	7.10	1.11	7.10	71.28*	42.47	0.804
NPT 33	15.19	5.74	21.71	8.80	0.73	4.32	23.43	41.55	0.814
NPT 34	12.86	6.33	24.76*	10.1	1.02	8.42	37.07	42.81	0.839
Mean	13.73	6.10	**20.24**	**8.63**	**0.92**	**7.62**	**57.51**	**41.03**	**0.886**
SEd	0.75	0.23	**1.58**	**0.67**	**0.04**	**0.87**	**2.74**	**0.08**	**0.002**
CD (5%)	1.54	0.48	**3.17**	**1.25**	**NS**	**1.74**	**5.48**	**NS**	**0.004**

Table.4 Genetic analysis of morphological, seed and biochemical traits

Characters	Coefficient of Variance		Heritability (%)	Genetic Advance
	Phenotypic (PCV)	Genotypic (GCV)		
Height	9.11	8.49	94	22.63
DBH	8.23	5.62	68	9.81
Canopy Height	6.31	5.12	81	18.53
Canopy Width	7.65	5.08	65	8.63
100 Seed Weight	22.31	21.95	98	23.93
100 Kernel Weight	33.87	32.63	96	23.08
Husk-Thickness	7.93	7.31	90	21.51
Decortication %	13.32	12.82	95	22.96
Moisture content %	12.37	12.24	98	23.47
Oil content %	7.11	6.85	91	28.86
Aza %	15.86	14.05	88	18.98

The seeds from various plus trees exhibited significant variability in seed and biochemical traits, which could be attributed to isolations that in turn influence of gene flow. Significant variability of seed characters *viz.,* seed size and weight was observed in selected plus trees (Bagchi and Sharma, 1989) and among various provenances of *Santalum album* (Veerendra *et al.,* 1999). This type of variability in seed morphology and germination was attributed to the out-breeding nature of sandalwood. Genetic control of seed size traits has been observed in several tree species like *Tectona grandis* (Jayasankar *et al*,. 1999), *Pongamia pinnata* (Sharma *et al.*, 2016) and *Bixa orellana* (Kala *et al.*, 2017)

Among seed and biochemical characters in 34 plus trees, the estimated genotypic coefficients of variation were less than that of the phenotypic coefficients of variation for all the characters. All characters expressed high heritability and medium genetic advance as percentage mean. The characters *viz.*, diameter at breast height (9.81%) and canopy width (8.63%) expressed low genetic advance as percentage mean (Table 4). A slight difference between phenotypic coefficient of variation and genotypic coefficient of variation and high estimation of heritability (broad sense) for all seed and biochemical traits under this study clearly revealed the heritable nature of variability present in plus trees. The genotypes coefficient of variation was more than that of the phenotypic coefficient of variation for all the characters indicating the influence of additive gene actions. Higher GCV indicates that worthwhile improvement could be achieved for this through simple selection. This result is concurrence with the finding of genetic parameters in *Azadirachta indica* (Kaushik *et al.*, 2005). High heritability and genetic gain observed for 100 seed weight (99.70%, 32.98 %), seed dye content (93.82 %, 29.05%) and seed bixin content (90.58%, 34.32 %) respectively indicated the additive gene actions (Kala *et al.*, 2017). High estimates of heritability (98%) have also envisaged that environment has comparatively very low influence on the seed traits and azadirachtin content. Heritability has an important place in tree breeding as it provides an index of the relative role of heredity and environment in the expression of various traits.

In conclusion, potentially huge genetic variability existed in seed and biochemical traits among the selected plus trees of *Azadirachta indica*. Among the 34, five plus trees *viz.*,NPT 11, NPT 22, NPT 17, NPT 4 and NPT 29 were found superior for all the traits studied including azadirachtin content. Hence, selection, mass propagation and popularization of these superior plus trees for industrial plantations would help to improve the overall productivity of neem in terms seed, oil and azadirachtin. Higher genotypic correlation coefficient of seed characters revealed that the traits are genetically controlled and selection can be very effective in further tree improvement programme.

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References

Bagachi, SK., (1995) Selection differential and predicted genetic gain in *Tectona grandis*. Ind. For. June: 482– 490

Bagchi, SK, and Sharma, VP., 1989. Biometrical studies on seed characters of *Santalum albu*m L. Silvae Genetica, 38(3-4): 152-153.

Burton, GW., 1952. Quantitative inheritance in grass. *Proc. Sixth Int.* Grassland Cong., 7: 277-283.

Devendra Kumar, 2015. Storage Response of Neem (*Azadirachta Indica* A. Juss.) Seed under Different Moisture and Temperature Regime. Global Journal of Science Frontier Research: C Biological Science, 15(6):1-18.

Diedhiou Djibril, Faye Mamadou, Vilarem Gérard, Mar-DiopCodouGeuye, Sock Oumar and Rigal Luc. 2015. Physical characteristics, Chemical composition and Distribution of constituents of the Neem seeds (*Azadirachta indica* A. Juss) collected in Senegal. Research Journal of Chemical Sciences, 5(7): 52-58.

Gera, M., Gera N and Ginwal HS 2000. Seed trait variations in *Dalbergia sissoo* roxb. Seed Sci.Technol. 28:467-475.

Girish, K., Bhat, S. and Shankara, 2008. Neem – A green treasure, Electronic Journal of Biology, 4(3): 102-111.

Jayasankar, S., Babu, LC, Sudhakara, K., and Kumar PD 1999. Evaluation of provenanced for seedling attributes in teak (*Tectona grandis* Linn. f.). Silvae Genetica, 48(3-4): 115-122.

Johnson, HW., Robinson HF and Comstock RE. 1955. Estimation of genetic and environmental variability in soya bean. Agron. J., 47: 314-318.

Kala, S., Kumaran, K., Srimathi, P., Reeja, S. and Anil Kumar, S. 2017. Studies on Variability, Correlation and Path Analysis Using Important Seed Traits in *Bixa orellana* (L). Journal of Tree Sciences, 36 (1): 93-102.

Kaushik, N., 2005. Inter and intra provenance variation in neem (*Azadirachta indica* A.Juss.) from different agroclimatic zones of haryana, *Ph.D. Thesis*, FRI, Deemed University, Dehra dun, India

Kaushik, N., 2002. Determination of azadirachtin and fatty acid methyl esters of *Azadirachta indica* seeds by HPLc and GLC Anal Bioanal Chem, 374:1199-1204.

Kaushik, N., 2002. Determination of azadirachtin and fatty acid methyl esters of *Azadirachta indica* seeds by HPLC and GLC. Anal. Bioanal. Chem., 374:1199–1204.

Kaushik, N., B. Gurudev Singh, U. K. Tomar, S. N. Naik, Satya Vir, S. S. Bisla, K. K. Sharma, S. K. Banerjee and Pramilla Thakkar, 2007. Regional and habitat variability in azadirachtin content of Indian neem (*Azadirachta indica* A. Jusieu). Current science 92(10):1400-1406.

Kumaran, K., 1997. Selection of superior one-parent families for higher growth, oil and azadirachtin contents in neem (*Azadirachta indica* A. Juss.), Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.pp.153.

Lush, KI., 1940. Intrasite correlation and regression of spering on dams as a method of establishing heritability of characters. *Proc. Amer. Soc. Animal Production,* 33: 293-301.

Martın, C., Moure, A., Giraldo Martın G., Carrillo E., Domınguez H. and Parajo J.C. 2010. Fractional characterization of Jatropha, Neem, Moringa, Trisperma, castor and candlenut seeds as potential feed stocks for biodiesel production in Cuba, *Biomass and Bioenergy*, 34: 533-538.

Panse, VG., and Sukhatme, PV., 1978. Statistical methods for Agricultural workers. ICAR Publication, New Delhi.

Sharma, S. S., Islam, M. A., Malik, A. A., Kumar, K., Negi, M. S., and Tripathi, S. B. (2016). Seed traits, fatty acid profile and genetic diversity assessment in *Pongamia pinnata* (L.) Pierre germplasm. *Physiology and molecular biology of plants: an international journal of functional plant biology*, *22*(2), 193-205.

Tiwari, D.N., 1992. Monograph on Neem (*Azadirachta indica* A. Juss). International Book Distributors, Dehradun, India.

Veerendra, HC, Ramalakshmi S and Mallesha BB, 1999. Variation in seed characters in provenances of sandal (*Santalum album* L.,). Ind. For.125:308-312

Willan, R.L., 1985. A guide to Forest seed handling with special reference to the tropics. FAO Forestry Paper 20/2. FAO, Rome

Yamasaki R.B., Klocke J.A., Lee S.M., Stone G.A. 1986. Isolation and purification of azadirachtin from neem (*Azadirachta indica*) seeds using flash chromatography and high-performance liquid chromatography. Journal of chromatography, 356: 220-226.

Zobel, B.,J and Talbert J 1984. Applied Tree Improvement. John Wiley & Co., Publication. New York. 503 p.

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