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Variation in seed morphology and selected oil parameters of neem (*Azadirachta indica* A. Juss.) from different agroclimatic zones in Tamil Nadu, India

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SUMMARY: Tamil Nadu, in southern India, has the second-largest number of neem trees in the country. The oil from the seeds has high economic significance for cottage industries in the region. This paper examines 28 Candidate Plus Trees (CPTs) selected from six agroclimatic zones in Tamil Nadu which exhibit exceptional traits such as superior growth and other desirable characteristics. We aimed to understand seed morphology variations and physicochemical properties in the oil across different regions. Significant differences were observed for morphometric traits. Fruit production correlated negatively with rainfall. 100-seed kernel weight and seed length correlated with oil percentage. Rainfall influenced seed breadth and pericarp weight. Clustering using morphological characters did not group genotypes from the same region; while soil type could distinguish them. Correlation helped us determine the prominent features which influence the traits of interest, which can be useful for breeding programs, cultivation practices, and the development of neem-based products in Tamil Nadu and beyond.

KEYWORDS: *Oil quality; Physical traits; Physicochemical properties; Variability.*

RESUMEN: ***Variación en la morfología de semillas y parámetros de aceites seleccionados de lilas india (*****Azadirachta indica** ***A. Juss.) de diferentes zonas agroclimáticas de Tamil Nadu, India.*** Tamil Nadu, en el sur de la India, tiene el segundo mayor número de árboles de lilas india del país. El aceite de las semillas tiene una gran importancia económica para las industrias artesanales de la región. En este trabajo se examinan 28 árboles Candidate Plus (CPT) seleccionados de seis zonas agroclimáticas en Tamil Nadu, que exhiben rasgos excepcionales como un crecimiento superior y otras características deseables. Nuestro objetivo era comprender las variaciones de la morfología de las semillas y las propiedades fisicoquímicas del aceite en diferentes regiones. Se observaron diferencias significativas para los rasgos morfométricos. La producción de frutos se correlacionó negativamente con las precipitaciones. El peso de 100 semillas y granos y la longitud de la semilla se correlacionaron con el porcentaje de aceite. Las lluvias influyeron en el ancho de la semilla y el peso del pericarpio. El agrupamiento utilizando caracteres morfológicos no agrupó genotipos de una misma región, mientras que el tipo de suelo pudo distinguirlos. La correlación nos ayudó a diseccionar las características prominentes que influyen en las características de interés, que pueden informar los programas de reproducción, las prácticas de cultivo y el desarrollo de productos a base de neem en Tamil Nadu y más allá.

PALABRAS CLAVE: *Calidad del aceite; Propiedades fisicoquímicas; Rasgos físicos; Variabilidad.*

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1. INTRODUCTION

Azadirachta indica A. Juss., (Neem; Family: Meliaceae), an evergreen, multipurpose tree which is native to India, grows in the arid, semi-arid, and tropical conditions in Pakistan, Bangladesh, Srilanka, Malaysia, Indonesia, Thailand, the Middle East, Sudan and Niger (Kaushik *et al.*, 2007). The species shows wide adaptability. It is seen as an avenue, ornamental agroforestry or roadside tree. It grows in clay, saline, alkaline, dry, stony, shallow soils, including high calcareous soil (Pattnaik *et al.*, 2006, Atabani *et al.*, 2013), and tolerates high temperatures, low rainfall, long spells of drought and salinity. For centuries, it has been used in traditional medicine. Various parts are used in the Ayurvedic and Unani systems of medicine. It is estimated that ~ 25 million neem trees exist in India, the highest recorded from Uttar Pradesh, followed closely by Tamil Nadu, Madhya Pradesh, Andhra Pradesh, and Karnataka. A fully grown tree produces about 50 kg fruits annually. The productivity of neem oil mainly varies from 2 to 4 t/ha/yr (Kaushik *et al.*, 2007).

Neem Seed Kernel Extract (NSKE) is widely used in agriculture. About 20–30% of the seed weight constitutes oil – the kernels contain 40–50% of an acrid green to brown oil (Atabani *et al.*, 2013). The oil comprises fatty acids, mainly oleic acid (13.50–26.76%), palmitic acid (6.88–11.99%), linoleic acid (7.32–11.17%), stearic acid (4.29–13.08%), and arachidic acid (0.53–1.27%) (Adigwe *et al.*, 2022). Azadirachtin, salannin, and other limonoids are also present in small quantities. Neem oil, being one of the least toxic to humans and beneficial organisms, is very promising in the control of numerous pests.

Kaushik *et al.* (2007) and Jessinta *et al.* (2014), provide information on the variation in tree morphology, vegetative and reproductive phenology, and seed morphology of the neem species across different geographic locations. However, there is a lack of information specifically related to agroclimatic zones. Tamil Nadu is the second in India's highest number of neem trees, so we felt it imperative to understand the variations across agro-climate zones. This study is significant considering the state's high number of neem trees and their relative abundance (12%), as reported by the Trees Outside Forests (TOF) inventory (FSI, 2019).

Hence, this paper discusses variations in seed morphology and seed oil properties in neem from various agroclimatic regions of Tamil Nadu. It would also enable us to predict site-source matched provenances for future planting.

2. MATERIALS AND METHODS

2.1. Collection of fruits

An extensive survey was carried out during 2019-20 in different agroclimatic regions of Tamil Nadu to select superior phenotypes of neem. Tamil Nadu has wide variations in its agro climatic zones (ACZ). The state is divided into seven ACZs. The present study covered six zones, leaving behind the Hilly region (Nilgiris), where neem trees are not present (FSI, 2019). The agroclimatic zones with precipitation and soil details are shown in Table 1. The details of selected Candidate Plus Trees (CPTs) concerning fruit yield, growth superiority, pest and disease incidence are presented in Table 2. The data on height, girth at breast height (GBH), clear bole height (CBH) and crown diameter were recorded and the seeding behavior of the selected CPTs was scored on a scale of 1 to 5 (1- Poor fruiting and 5 - Heavy fruiting). Fully mature, yellowish-green fruits were collected from the CPTs. Within 24 hours of collection, the fruits were de-pulped and their seeds were washed thoroughly with tap water to remove pulp, dirt and impurities. The seeds were shade-dried and stored under ambient conditions. All experiments were conducted in a completely randomized design with four replicates.

2.2. Measurement of seed morphometric characters

Neem seeds (100 in four replicates – 25 in each) were spread on the glass plate of a macro viewer and images were captured. Using Leica Q Win, the length (cm) and breadth (cm) of the seeds were recorded. The 100-seed weight (g), 100-seed kernel weight (g) and pericarp weight (g) of the seeds were estimated using an electronic balance and pericarp percentage was calculated from the recorded values. The moisture content of the neem seeds was estimated as per ASAE (1998).

2.3. Oil extraction and characterization

Twenty grams of seed kernel powder were used for oil extraction. The oil content was extracted using the standardized Soxhlet method. Petroleum ether was used as solvent.

TABLE 1. Agroclimatic zones (ACZ) with the number of trees selected in parenthesis, along with precipitation and soil details

ACZ No	Agroclimatic zones	Locations	Minimum Annual Rainfall (mm)	Maximum Annual Rainfall (mm)	Total Annual Rainfall (mm)	Soil Type
1	Western (15)	Coimbatore	121.8	673.6	1206.1	Red or Black Loam
		Tiruppur	20.5	146.9	600.3	
		Erode	28.7	212.1	653.4	
2	NorthEastern (1)	Kallakurichi	36.3	278.5	860.1	Red clay loam
3	High Rainfall (3)	Thoothukudi	5.7	61.8	593.1	Saline Coastal
4	NorthWestern (2)	Salem	54.3	365.1	850.8	Non-Calcareous red and brown
5	Cauvery Delta (1)	Trichy	26.3	242.2	715.8	Red Loamy or Alluvium
6	Southern (9)	Ramnathapuram	14.1	113.0	805.3	Red Sandy
		Tirunelveli	26.9	135.6	841.8	

Source: District Statistics, TN (2020)

TABLE 2. Variation in tree morphometric traits in the selected Candidate Plus Trees (CPTs) of *Azadirachta indica.*

ACZ	Accession code	Tree height (m)	Girth at Breast Height (cm)	Clear Bole Height (m)	Crown diameter (m)	Seeding behavior
ACZ1	IFGTB AI 1	12.16	150	1.3	11.2	5
ACZ1	IFGTB AI 2	13.28	155	0.85	10.56	4
ACZ1	IFGTB AI 3	13.06	148	1.2	12.10	4
ACZ1	IFGTB AI 4	12.46	151	1	13	3
ACZ1	IFGTB AI 6	13.12	136	1.62	10.60	4
ACZ1	IFGTB AI 9	11.10	142	1.30	10.89	4
ACZ1	IFGTB AI 10	14.23	155	1.85	13.5	3
ACZ1	IFGTB AI 12	11.56	126	1.32	12.16	4
ACZ1	IFGTB AI 13	13.65	134	1.25	14.18	4
ACZ1	IFGTB AI 14	14.12	128	1	10.10	4
ACZ1	IFGTB AI 16	12.10	145	1.32	13.36	3
ACZ1	IFGTB AI 17	11.22	123	1.23	14.65	4
ACZ2	IFGTB AI 18	13.08	169	1.65	15.11	4
ACZ3	IFGTB AI 19	10	135	1.11	10.0	5
ACZ3	IFGTB AI 20	9.80	110	1.21	10.48	5
ACZ3	IFGTB AI 21	14.12	130	1.5	16.15	5
ACZ4	IFGTB AI 22	12.28	142	1.33	11.34	4
ACZ4	IFGTB AI 23	13.36	132	1.62	12.25	5
ACZ5	IFGTB AI 28	16.11	186	1.25	18.12	4
ACZ6	IFGTB AI 29	8.98	110	1.36	10	4
ACZ6	IFGTB AI 31	15.50	168	1.22	17.10	4
ACZ6	IFGTB AI 32	17.22	185	1.14	20.42	3
ACZ6	IFGTB AI 33	10.15	125	1.19	10.23	3
ACZ6	IFGTB AI 34	13.15	129	1.23	15.18	4
ACZ6	IFGTB AI 37	12.18	116	1.54	12.13	4
ACZ1	IFGTB AI 38	15.50	128	1.65	16.26	4
ACZ1	IFGTB AI 40	10.48	130	1.10	13.15	4
ACZ1	IFGTB AI 41	11.24	126	1.05	12.22	4

ACZ: Agroclimatic Zone; IFGTB AI: Institute of Forest Genetics and Tree Breeding *Azadirachta indica*

2.3.1. Physical properties

Oil color was determined visually and the odor was determined by the volatilized smell. The pH of the oil samples was analyzed using pH indicator strips (Merck, Germany). The refractive index (RI) of the oil was estimated using the standard method of AOAC (2007) and quantified using a pen Refractometer (Atago, Japan) with a resolution and accuracy value of ± 0.1 and ± 0.2%, respectively at 10-60

°C. The specific viscosity of the oil was measured according to the standard method ASTM (2003) at 30 °C using the Engler Viscometer and expressed in Degrees Engler (°Engler).

2.3.2. Chemical properties

Acid value. The acid value was determined according to the method described by AOAC (2007) and expressed as the KOH (in mg) necessary to neutralize the free fatty acids contained in 1 g of oil.

Saponification value. A measurement of the fatty acid chain length in oils was determined by standard procedures (AOAC, 2007) and expressed in milligrams KOH absorbed per gram of oil.

Iodine value. The iodine value was estimated by Oomah *et al.* (2000).

Peroxide value. The peroxide value was determined by Cox and Pearson (1962) and expressed in meq $O_2 \cdot kg^{-1}$ of oil.

Specific gravity. Specific gravity was measured following the standard method of AOCS (1997).

2.4. Statistical analysis

A statistical analysis was conducted using SPSS (v. 20). Analysis of variance followed by post-hoc (Duncan's Multiple Range Test (DMRT)) was performed at a 5% significance level. Correlation analyses were employed to find the relationship between the meteorological data and seed morphology with the oil contents. The dendrogram was constructed by the Ward method of cluster analysis on the Average distances between zones. To determine the robustness of the dendrogram, the data was bootstrapped with 1000 replicates.

3. RESULTS

3.1. Variations in plus trees and morphometric characteristics of the seeds

The tree height of the individual trees varied from 8.98 m in IFGTB AI 29 to 17.22 m in IFGTB AI 32; whereas the highest GBH was recorded in IFGTB AI 28 (186 cm) and the lowest value in IFGTB AI 29 (110 cm) (Table 2). Variations were also observed among the different ACZs. The GBH and seeding behavior among the ACZs showed significant ($P < 0.05$) variations. The highest GBH was recorded in ACZ 5 (186 cm), while ACZ 3 recorded the lowest (125 cm) values. Seeding behavior was highest in ACZ 3 and lowest in ACZs 1 and 6. A strong (0.531) negative correlation was observed between seeding behavior and total annual rainfall (data not shown), indicating the influence of yearly rainfall on seeding behavior. Crown diameter, however, did not show any relation to seeding behavior.

Significant variations were recorded among the 28 Candidate plus trees for all seed characters (Table 3). The highest seed length was recorded in IFGTB AI-1 (2.11 cm) followed by IFGTB AI 38 (2.07 cm); whereas the lowest values were registered for IFGTB AI 33 (1.05 cm). Seed breadth was the highest in IFGTB AI 41 (1.09 cm) followed by IFGTB AI 38 (0.91cm) and lowest in IFGTB AI 20 (0.56cm). The 100-seed weight varied from 10.14 g in IFGTB-17 to 36.38g in IFGTB AI - 1. The highest hundred-kernel weight of 19.93 g was recorded for IFGTB AI- 1 followed by 15.44 g for IFGTB AI - 38. The pericarp percentage showed significant differences among all the plus trees. Three plus trees viz., IFGTB AI 14 (60%), IFGTB AI 22 (59.46%) and IFGTB AI 23 (59.02%) showed superiority in pericarp percentage. IFGTB AI-41 recorded the highest pericarp weight (17.25 g) and the lowest (5.12 g) was recorded for IFGTB AI 20. The maximum kernel weight was recorded for IFGTB AI 1 (19.93 g) and the minimum was for IFGTB AI 40 (5.18 g). (Table 3). The oil content of neem seed kernels exhibited large variations (25.06 to 45.45%) in different zones (Table 3). The maximum seed oil content was recorded for IFGTB AI 1 (45.45%) followed by IFGTB AI 38 (43.96%) and the minimum percentage was recorded for IFGTB AI 33 (25.06 %).

There were significant ($P < 0.05$) variations among the ACZs as well. ACZ5 recorded high values for seed length, 100-seed and kernel weight and oil percentage. However, ACZ 4 showed high values for breadth, pericarp weight and pericarp percentage (Table 4), suggesting fruits with thicker pericarps.

The correlation matrix of seed characteristics, oil content and rainfall are given in Table 5. Seed morphometric characteristics such as 100-seed weight ($r = 0.959$), 100-kernel weight ($r = 0.848$) and seed length ($r = 0.874$) recorded strong positive correlation with oil percentage of neem seeds. However, the correlation between seed breadth and oil percentage was negligible ($r = 0.08$). Rainfall influenced seed breadth ($r = 0.311$) and pericarp weight ($r = 0.308$). The total minimum and maximum rainfall influenced pericarp percentage (Table 5).

TABLE 3. Variations in the morphological characteristics of Neem seeds collected from different parts of Tamil Nadu

S.No.	Candidate Plus Trees	Seed Length (cm)	Seed Breadth (cm)	100-Seed weight (g)	100-Seed kernel weight (g)	Pericarp weight (g)	Pericarp (%)
1	IFGTB AI 1	2.11	0.68	36.38	19.93	8.2	46.25
2	IFGTB AI 2	1.56	0.69	20.64	9.47	8.2	45.57
3	IFGTB AI 3	1.69	0.7	26.72	13.95	9.45	55.65
4	IFGTB AI 4	1.45	0.66	21.68	9.76	7.62	47.19
5	IFGTB AI 6	1.37	0.65	17.77	9.55	6.23	52.99
6	IFGTB AI 9	1.5	0.64	16.19	8.97	6.79	39.23
7	IFGTB AI 10	1.46	0.59	17.32	12.95	5.98	47.82
8	IFGTB AI 12	1.59	0.66	25.06	9.6	7.35	42.30
9	IFGTB AI 13	1.53	0.58	17.67	8.97	5.16	50.69
10	IFGTB AI 14	1.32	0.84	13.92	6.48	10.32	59.78
11	IFGTB AI 16	1.54	0.67	22.12	11.96	7.94	56.84
12	IFGTB AI 17	1.23	0.65	10.14	4.98	6.8	44.38
13	IFGTB AI 18	1.52	0.67	16.93	8.47	7.28	54.71
14	IFGTB AI 19	1.45	0.65	16.52	8.6	7.06	42.54
15	IFGTB AI 20	1.55	0.56	25.11	12.95	5.12	14.55
16	IFGTB AI 21	1.6	0.66	21.88	10.95	7.81	43.79
17	IFGTB AI 22	1.38	0.88	15.27	7.47	11.55	59.24
18	IFGTB AI 23	1.5	0.8	18.62	10.5	11.1	58.80
19	IFGTB AI 28	1.85	0.68	26.89	14.95	9.16	48.99
20	IFGTB AI 29	1.35	0.73	19.28	10.46	10.34	44.26
21	IFGTB AI 31	1.65	0.74	17.26	13.95	10.88	48.23
22	IFGTB AI 32	1.54	0.76	22.99	9.96	10.75	58.74
23	IFGTB AI 33	1.05	0.65	11.71	5.48	7.54	32.69
24	IFGTB AI 34	1.32	0.84	13.26	6.75	12.65	47.97
25	IFGTB AI 37	1.29	0.78	12.83	5.98	11.75	47.62
26	IFGTB AI 38	2.07	0.91	26.9	15.44	11.2	52.28
27	IFGTB AI 40	1.28	0.72	12.46.	5.18	12.12	44.68
28	IFGTB AI 41	1.34	1.09	16.09	8.47	17.25	45.95
	Mean	1.50	0.71	19.52	10.07	9.05	47.63
	SEd	0.07	0.03	0.89	0.47	0.43	2.15
	Critical Difference	0.09	0.04	1.26	0.66	0.61	3.04

*Values are means of four replicates. IFGTB AI: Institute of Forest Genetics and Tree Breeding *Azadirachta indica.*

TABLE 4. Variations in morphometric traits in *Azadirachta indica* seeds from different agro-climatic zones (ACZ). Means compared using Duncan's Multiple Range Test (DMRT).

ACZ	Seed Length (cm)	Seed Breadth (cm)	100-Seed weight (g)	100-Seed kernel weight (g)	Pericarp weight (g)	Pericarp (%)	Oil (%)
ACZ1	1.54 ± 0.26b	0.72 ± 0.14ab	20.07 ± 6.65b	10.38 ± 3.91b	8.71 ± 3.03ab	48.77 ± 6.13b	33.89 ± 6.34b
ACZ2	1.52 ± 0.09b	0.67 ± 0.04b	16.93 ± 1.02b	8.47 ± 0.51b	7.28 ± 0.44b	54.71 ± 3.28ab	30.46 ± 1.83b
ACZ3	1.53 ± 0.1b	0.62 ± 0.06b	21.17 ± 3.92ab	10.83 ± 1.97b	6.66 ± 1.25b	33.63 ± 14.44c	33.99 ± 4.29b
ACZ4	1.44 ± 0.1b	0.84 ± 0.06a	16.95 ± 2.05b	8.99 ± 1.73b	11.33 ± 0.66a	59.02 ± 3.18a	30.57 ± 3.13b
ACZ5	1.85 ± 0.11a	0.68 ± 0.04b	26.89 ± 1.61a	14.95 ± 0.9a	9.16 ± 0.55ab	48.99 ± 2.94b	43.2 ± 2.59a
ACZ6	1.37 ± 0.21b	0.75 ± 0.07ab	16.22 ± 4.22b	8.76 ± 3.12b	10.65 ± 1.72a	46.59 ± 8.23b	29.25 ± 4.01b

*Values are means of four replicates. Means were compared using DMRT. The values denoted by a different letter indicate significant differences among treatments (**$p < 0.05$**).

TABLE 5. Correlation studies of seed parameters and oil content in *Azadiracta indica* with precipitation

Correlations	Seed Length	Seed Breadth	100-Seed Weight	100-Kernel Weight	Pericarp Weight	Pericarp %	Minimum Annual Rainfall	Maximum Annual Rainfall	Total Annual Rainfall	Oil %
Seed Length	1	0.065	.860**	.873**	-0.051	0.169	0.113	0.154	-0.128	.874**
Seed Breadth		1	-0.106	-0.094	.903**	.422**	0.148	0.141	.311**	-0.08
100-Seed Weight			1	.892**	-0.143	0.019	0.064	0.092	-0.198	.959**
100-Kernel Weight				1	-0.121	0.039	0.032	0.059	-0.164	.848**
Pericarp Weight					1	.368**	0.027	0.015	.308**	-0.144
Pericarp %						1	.418**	.444**	.535**	0.072
Minimum Annual Rainfall							1	.985**	.647**	0.115
Maximum Annual Rainfall								1	.609**	0.153
Total Annual Rainfall									1	-0.176
Oil %										1

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

TABLE 6. Variations in physical and chemical properties of Neem oil extracted from seeds collected from different parts of Tamil Nadu

S.No.	Candidate Plus Trees	Specific gravity ($g \cdot ml^{-1}$)	Refractive Index	Viscosity ($mm^2 \cdot s^{-1}$)	Moisture (%)	pH	Oil percentage	Peroxide value (meq $O_2 \cdot kg^{-1}$ oil)	Saponification value (mg $KOH \cdot g^{-1}$)	Iodine value ($gI \cdot 100\ g^{-1}$)	Acid value (mg $KOH \cdot g^{-1}$)
1	IFGTB AI 1	0.80	1.66	44.73	14.70	4.19	45.45	12.28	167.67	39.45	20.13
2	IFGTB AI 2	0.83	1.45	45.33	1.70	4.20	33.86	10.20	234.74	52.09	25.71
3	IFGTB AI 3	0.82	1.46	49.02	3.84	4.34	40.9	11.40	190.74	40.96	26.83
4	IFGTB AI 4	0.79	1.67	51.21	0.60	4.79	34.13	10.68	245.91	41.98	22.36
5	IFGTB AI 6	0.59	1.62	54.00	3.45	5.03	32.06	13.23	190.03	27.82	27.95
6	IFGTB AI 9	0.85	1.66	45.23	3.53	4.38	29.4	13.07	190.03	37.93	21.24
7	IFGTB AI 10	0.75	1.46	53.80	1.10	4.82	31	8.37	312.98	42.48	22.36
8	IFGTB AI 12	0.81	1.65	44.54	1.57	4.18	40.8	12.20	122.96	26.8	18.99
9	IFGTB AI 13	0.75	1.65	45.33	0.76	4.35	33.33	12.28	190.03	24.59	21.24
10	IFGTB AI 14	0.81	1.66	53.30	0.92	4.75	27.9	11.56	212.38	63.21	26.83
11	IFGTB AI 16	0.80	1.46	54.10	1.13	4.05	34.53	12.04	245.91	47.03	26.83
12	IFGTB AI 17	0.75	1.65	47.42	3.17	4.32	26	7.81	234.74	60.68	24.59
13	IFGTB AI 18	0.76	1.65	49.71	0.70	4.57	30.46	10.76	299.1	65.23	21.24
14	IFGTB AI 19	0.70	1.65	51.51	0.80	4.20	29.4	7.25	279.45	54.11	25.71
15	IFGTB AI 20	0.77	1.65	54.60	1.51	4.56	38.4	10.04	201.2	64.23	29.06
16	IFGTB AI 21	0.81	1.65	54.10	3.79	4.81	34.17	10.84	223.56	60.68	22.36
17	IFGTB AI 22	0.91	1.64	51.71	0.60	4.51	28.13	11.40	290.63	55.12	25.71
18	IFGTB AI 23	0.83	1.64	53.50	2.58	4.12	33	7.33	223.56	63.21	24.59
19	IFGTB AI 28	0.80	1.64	51.21	2.21	4.90	43.2	8.69	201.20	52.59	24.59
20	IFGTB AI 29	0.79	1.65	55.19	6.48	5.43	31.26	7.01	312.98	66.75	31.3
21	IFGTB AI 31	0.78	1.64	49.02	8.65	5.28	30.23	9.96	268.27	66.25	25.71
22	IFGTB AI 32	0.80	1.64	50.31	6.46	4.07	35.73	7.41	297.91	39.95	29.06
23	IFGTB AI 33	0.79	1.64	51.91	2.10	4.19	25.06	10.60	212.38	50.57	25.71
24	IFGTB AI 34	0.86	1.64	58.18	6.39	5.50	26.8	9.80	279.45	67.77	37.77
25	IFGTB AI 37	0.81	1.65	58.88	1.88	5.40	26.4	3.35	319.54	67.26	33.54
26	IFGTB AI 38	0.83	1.64	57.18	2.50	5.22	43.96	6.94	299.89	64.23	26.83
27	IFGTB AI 40	0.84	1.65	54.60	1.28	4.54	26.26	11.88	245.91	64.23	29.06
28	IFGTB AI 41	0.68	1.65	57.29	1.99	5.45	28.8	5.58	312.98	70.3	38.01
	Mean	0.78	1.62	51.67	3.08	4.64	32.87	9.78	243.07	52.76	26.26
	SEd	0.03	0.07	2.30	0.19	0.20	5.77	0.44	10.90	2.41	1.17
	Critical Difference	0.05	0.10	3.25	0.27	0.29	15.17	0.63	15.42	3.41	1.81

*Values are means of four replicates. IFGTB AI: Institute of Forest Genetics and Tree Breeding *Azadirachta indica.*

3.2. Physical properties

The physical properties of neem seed oil across the plus trees (Table 6) and zone-wise (Table 7) reveal significant differences only in specific gravity. No significant differences were observed among trees within the same zone. The highest specific gravity was observed in oil from ACZ4 (0.87) and the lowest in ACZ 3 (0.72). The viscosity of the neem oil in zones ranged from 44.8 to 53.59 $mm^2·s^{-1,}$ with values for individual trees ranging between 44.54 $mm^2·s^{-1}$ and 58.88 $mm^2·s^{-1}$. The oil's refractive index was the highest in IFGTB AI 4 (1.67) and the lowest in IFGTB AI 2 (1.45). The average pH in the seed oil was 4.64 and the maximum was recorded for IFGTB AI 34 (5.5) and minimum for IFGTB AI 16 (4.05) (Table 6).

3.3. Chemical properties

The chemical properties of neem seed oil are shown in Table 7. Except for peroxide values, all other chemical properties were significantly different ($P < 0.05$) across zones and among chemical properties (Table 7). Zone ACZ 2 recorded high values for both saponification and iodine values. ACZ 6, 5 and 3 recorded high values for saponification, acid and peroxide values, respectively. The highest saponification value in the oil was obtained for IFGTB AI 37 (319.54 mg $KOH·g^{-1}$), followed by IFGTB AI 10, IFGTB AI 29 and IFGTB AI 41 (312.98 mg $KOH·g^{-1}$), and the lowest was detected in IFGTB AI 12 (122.96 mg $KOH·g^{-1}$) (Table 6). The acid value of the oil was found to be at its highest in IFGTB AI 41 (38.01 mg $KOH·g^{-1}$) and its lowest in IFGTB AI 12 (18.99 mg $KOH·g^{-1}$). The highest iodine value in the oil was found for IFGTB AI 41 (70.3 $gI·100\ g^{-1}$), followed by IFGTB AI 34 (67.77$g·ml^{-1}$), with the minimum value recorded for IFGTB AI 13 (24.59 $gI·100\ g^{-1}$) (Table 6).

Clustering revealed that neem from ACZ 3 was distinct from the rest of the zones. In the second clade, neem from ACZ 5 was clustered separately. ACZs 1 and 6 were grouped together. Likewise, ACZs 2 and 4 were grouped together.

4. DISCUSSION

Cataloguing seed morphological features from natural populations is considered the first step in understanding the genetic variability of a species. Multiple factors could induce and maintain variation in seed features. Large seeds are favored as they produce large and vigorous seedlings. On the contrary, smaller seeds may have a better selection advantage due to broader and more effective dispersal (Eriksson 1999). Fruit and seed characteristics, namely weight, length, width, diameter, yield and oil content, are reported highly variable both within and among the provenances of neem (Kundu and Tigerstadt, 1998; Jindal *et al.*, 1999). The present study also revealed significant variation among individual trees (CPTs) and among agroclimatic zones. Kundu and Tigerstadt (1997) reported distinct clustering of neem provenances based on rainfall regions. A strong negative correlation was observed in the present study between seeding behavior and total annual rainfall, in line with earlier reports. Thus, it could be concluded that rainfall plays a crucial role in seed-bearing.

Seeds are influenced by various factors, such as geographic area, climate, genetic variability, agronomic conditions, plant morphology and physiology,

Table 7. Physical and chemical properties of neem seed oil from different agro-climatic zones (ACZ). Means compared using DMRT.

ACZ	Specific gravity	Refractive Index*	Viscocity* (mm^2/s)	pH*	Peroxide value* (meq $O_2·kg^{-1}$ oil)	Saponification value (mg $KOH·g^{-1}$)	Acid value (mg $KOH·g^{-1}$)	Iodine value ($g·ml^{-1}$)
ACZ1	0.79 ± 0.09ab	1.60 ± 0.17	52.67 ± 6.36	4.74 ± 0.62	9.99 ± 2.62	240.71 ± 53.48abc	26.5 ± 4.94b	47.99 ± 14.07b
ACZ2	0.76 ± 0.08ab	1.65 ± 0.18	44.80 ± 4.81	4.58 ± 0.49	10.78 ± 1.16	299.60 ± 32.16a	20.16 ± 2.16c	66.36 ± 7.12a
ACZ3	0.72 ± 0.08b	1.65 ± 0.15	52.03 ± 5.29	4.52 ± 0.5	8.09 ± 3.66	197.81 ± 70.8bc	24.26 ± 4.32bc	59.77 ± 7.13ab
ACZ4	0.87 ± 0.09a	1.64 ± 0.16	52.99 ± 5.25	4.32 ± 0.47	11.86 ± 1.24	257.52 ± 44.45ab	23.51 ± 3.34bc	66.87 ± 7.51a
ACZ5	0.80 ± 0.09ab	1.64 ± 0.18	53.59 ± 5.75	4.91 ± 0.53	8.70 ± 0.93	190.35 ± 20.43c	38.07 ± 4.09a	52.68 ± 5.65ab
ACZ6	0.81 ± 0.08ab	1.65 ± 0.15	49.81 ± 6.13	4.61 ± 0.76	9.53 ± 2.07	268.07 ± 43.33a	26.50 ± 3.37b	54.68 ± 15.99ab

*Values are means of four replicates. Means were compared using DMRT. The values denoted by a different letter indicate significant differences between treatments ($p < 0.05$). *Not Significant collection and storage of plant material (Fernandes *et al.*, 2019). Though our reports are consistent with reports on variability in seed length in neem seeds collected from five provenances in northern and western India (Kaura *et al.*, 1998), the 100-seed weight ranged from 10.14 to 36.38 g, while they report 0.8 to 3.5 g, indicating smaller seeds. Variation in seed parameters such as seed diameter, seed length, kernel-to-seed ratio, 100-kernel weight and 100-seed weight have also been reported by Gupta *et al.* (2012) in different provenances in Gujarat. Kumaran *et al.* (1993) reported high heritability for seed length, seed oil content, and 100-seed weight. These parameters could be a robust selection index for neem.

We also obtained significant correlations between oil content and 100-seed and kernel weight in accordance with their findings. Neem seeds collected from different locations in Tamil Nadu showed a positive correlation between oil content and the number of hours of sunshine (Sridharan *et al.*, 1998). As presented in Table 4, rainfall influenced seed breadth, pericarp weight and percentage, suggesting that rounder fruits with thicker pericarps could be observed in high rainfall areas. Seed and kernel weight, which positively correlated with oil content, can be considered promising traits for the early selection of seed sources. Similar results have been reported for other tree-borne oil seeds (Kaura *et al.* 1998).

Seed oil content varies among tree-borne oil seeds (Vollmann *et al.* 2007). Oil yield is also affected by tree age, seed extraction method, seed storage, and environmental factors. This variation in the present study and other seed morphological attributes presents us with a viable selection alternative from base seed material at a very early stage. This could be useful for the improvement of programs, especially considering that the neem is commercially important for its oil and azadirachtin contents. We recorded 14 to 60% oil content in neem seed kernels, similar to Kaura *et al.*, (1998) and Tomar *et al.* (2011), who report the influence of agroclimatic zones on oil content.

The present study recorded the highest oil percentage from ACZ 5, comprising alluvial soil, which is rich in minerals, especially potash. This may have contributed to its high oil content, as indicated by Devaranavadagi *et al.* (2003), who reported oil content variation due to climatic and site conditions. Sidhu *et al.* (2003) also reported low oil contents in neem from arid, saline and coastal regions.

The Bureau of Indian Standards (BIS) IS 4765: 1975 Specification for Neem Kernel Oil and Depulped Neem Seed Oil (Reaffirmed in 2018) prescribes a specific gravity range of 0.908-0.934 for neem oils. None of the samples fell within this range. The BIS also defined ranges for saponification value (188-205 mg KOH$\cdot$g^{-1}), iodine value (65-80 gI$\cdot$100 g^{-1}) and acid value (15 mg KOH$\cdot$g^{-1}).

Acid value is a relative measure of rancidity as free fatty acids, while iodine value defines the drying quality of the oil. The low iodine values obtained for the samples studied represents the fewer unsaturated bonds, indicating that the low tendency of the oil to undergo oxidative rancidity. Accordingly, the peroxide value, an indication of the rate of rancidity was also low (Table 7). The presence of water or moisture contributes to hydrolysis, thus leading to higher acid values, and reducing the storage capacity of the oil (Do *et al.*, 2022).

The saponification value, a measure of the average molecular weight of all the fatty acids in the sample in triglycerides, varied from 190.35 to 299.6 mg/KOH. Hussein *et al.* (2021) also reported a wide range of variation in neem. A higher acid value increases saponification value (Hussein *et al.*, 2021), thus increasing the possible utilization of the oil in soaps and cosmetics. The proportion of each fatty acid in the oil may vary from tree to tree because of genetic make-up. A high saponification value implies the potential tendency to soap formation, and long-stored degraded oils are good for soaps and toiletry product productions (Hussein *et al.*, 2021). The prevalence of a wide variation in the neem accessions collected from different agroclimatic zones helped us classify the oil's utility prospects from the different agro-climatic zones. Genotypes with higher saponification values could be recommended for the cosmetic industry.

Morphometric techniques are valuable tools for exploring population differentiation, allowing more rigorous comparisons within a genus (Kolawole *et al.* 2016). Morphological characterization reveals diversity between germplasm. The clustering of zones based on seed morphology and oil content revealed that soil played a significant role in grouping the accessions. The saline coastal (ACZ 3) and rich alluvial (ACZ 5) zones remained distinct. However, it is to be noted that distinction based on morphological characteristics may not cluster genotypes from the

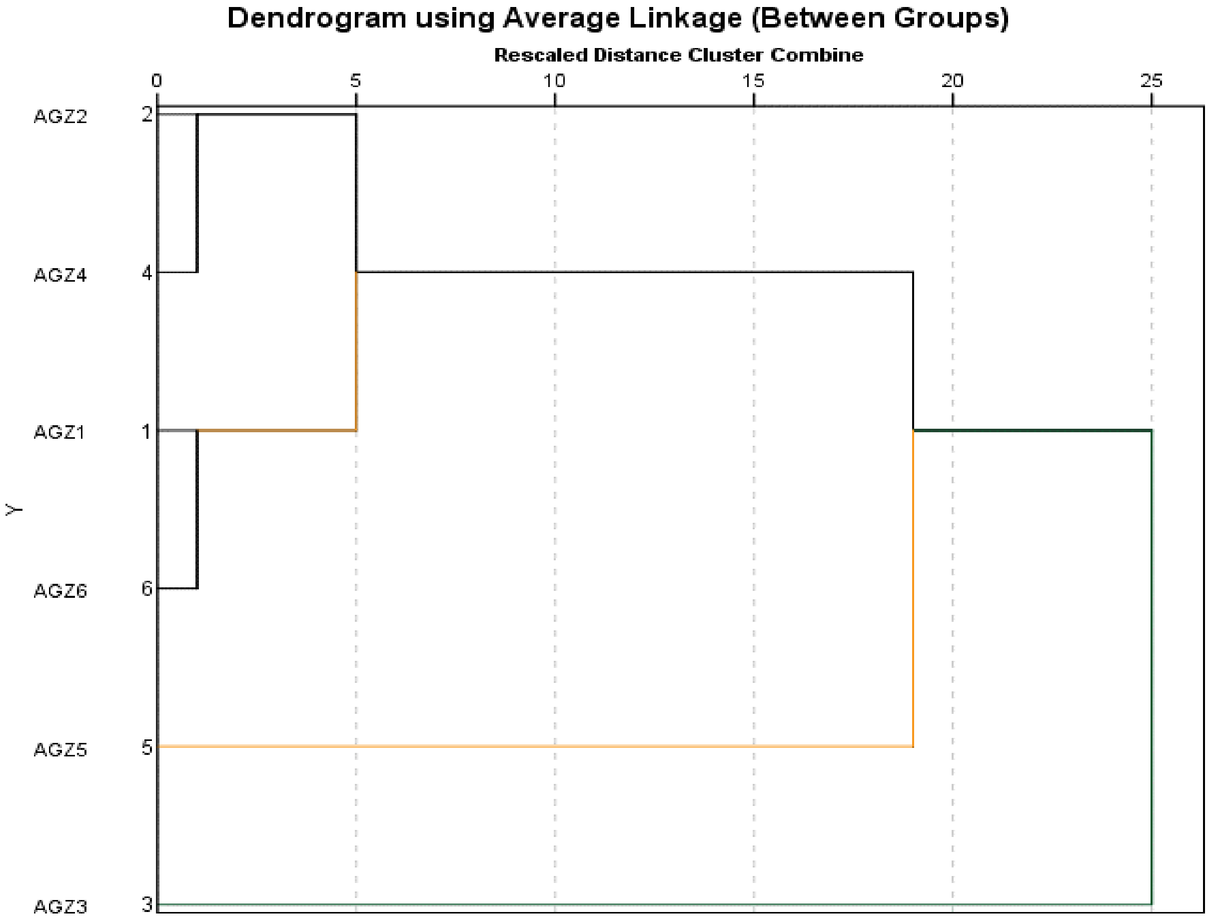

FIGURE 1. Dendrogram of neem from different Agro-climatic Zones (ACZ) studied based on Ward method using Average linkage.

same region within the same group (Figure 1). However, Kolawole *et al.* (2021) suggested phenetic dendrograms as the first step to grouping variables, dissecting the prominent features which influence the traits of interest, followed by a rigorous selection.

Understanding the variations in seed morphology and seed oil properties across agroclimatic zones allows for a comprehensive understanding of the species' adaptability and response to different climatic conditions. This knowledge can aid in selecting appropriate neem provenances for future planting in specific agroclimatic regions. Variations in seed morphology and seed oil properties have implications for various applications of neem products. Neem oil, derived from the seeds, is widely used in agriculture, medicine, cosmetics, and other industries. The quality and composition of neem oil is observed to vary based on geographical factors, which in turn affects its efficacy and suitability for different purposes.

Studying the seed morphology and seed oil properties across various agroclimatic regions in Tamil Nadu provided insights into the geographic variations within the neem species. This information contributes to the development of site-source matched provenances, helping in the selection of neem trees that are well-suited to specific agroclimatic conditions.

5. CONCLUSIONS

Cottage industries in Tamil Nadu extensively utilize neem for the production of various products. Neem oil, extracted from neem seeds, is a primary ingredient in the manufacturing of soaps, shampoos, hair oils, and other personal care items. Neem-based products from Tamil Nadu's cottage industries have the potential for export, contributing to the state's economy. With a wide distribution of the species in the state, assessing the extent of variability becomes pertinent. This paves the way for developing a defined tree program by targeting oil. Soil type and rainfall influenced oil content. Of the twenty-eight superior trees identified from six agro-climatic zones, the oil content was the highest in those growing in alluvial soil. Fruit yield was also dependent on rainfall. Information on the influence of environmental variables is crucial to identify high oil-yielding populations. This would enable the selection of genotypes for developing improved and adapted cultivars. Superior genotypes from these selections can also be used for establishing large scale plantations.

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