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Evaluation of Azadirachtin Content of Neem (*Azadirachta indica* A. Juss) Seeds from Different Provinces of India

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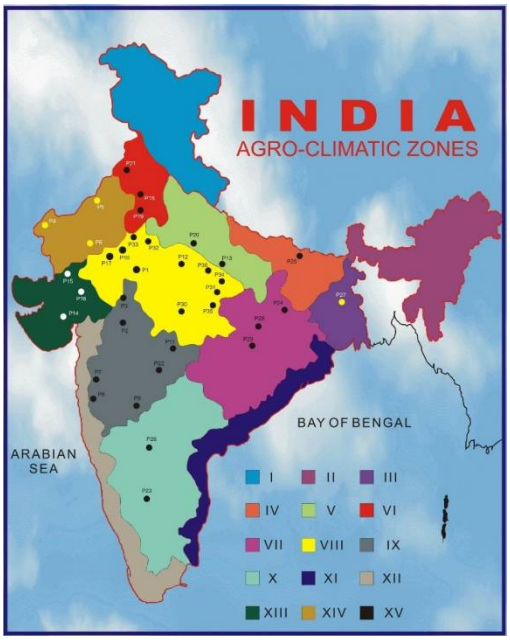
ABSTRACT

Azadirachta indica is a pesticidal plant widely used for plant protection. In the present study, seeds were collected from 36 provinces which were covered 10 seed zones of India. The concentration of Azadirachta in content varied from 0.11 to 0.91% with an average value of 0.27% by weight of neem seed kernels. Hence, the present study would be helpful for seed collection and raising of azadirachtin rich germplasm bank for future investigations.

Graphical Abstract



Neem Province from different Agro climatic Zones of India

Keywords: Neem, Provinces, Azadirachtin, NSK.

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 subcontinent since antiquity. Neem extracts and compounds have attracted the special interest of entomologist and phytochemists all over the world [**1-3**]. These extracts possess insect repellents, antifeedants, growth inhibitors and other insecticidal properties [**2, 4, 5**]. Neem seed kernel (NSK) is the most important source of triterpenoids [**6, 7**] and azadirachtin (Az) is considered to be the compound of the most biological interest [**1, 4, 8**]. Azadirachtins are steroid-like tetranotriterpenoids formed by a group of closely related isomers, called Azadirachtin-A to Azadirachtin-G [**9, 10**]. Several other active compounds were isolated from NSKs, such as salannin, gedunin and nimbin [**11**]. Az is a highly oxidized triterpenoid and one of the most potent antifeedant compounds yet discovered [**12**] and it affects the normal growth and development of a wide spectrum of insects [**2, 13**]. However, its lability to heat, moisture, air, etc. has been a matter of concern that led to global efforts to stabilize it [**14-17**]. Az represents about 0.2-0.8% of the seeds by weight, and it is accompanied by a number of other triterpenoids, such as nimbin [**18**] and salannin [**19**], which exhibit similar biological properties, to a greater or lesser extent. The amount of Az may vary considerably, depending on environmental and genetic factors. Az varies greatly among different trees and regions or countries [**20-22**]. Samples from 22 countries were investigated by Ermel [**20**] for more than 4 years showed marked variations between samples from different countries and among samples from the same country with mean value of 3.6 mg g^{-1} of Az and 46.7% neem oil (N.O.). The highest content of Az (10 mg g^{-1} seed kernel) was recorded by Shaun et al. (1996) in newly ripened seeds; there was some loss of Az and salannin in storage after harvesting for up to 6 months. Az- A is the currently accepted reference ingredient for standardizing neem-based products [**23**]. Synthetic pesticides possess quick knock down effect, but they are often toxic to mammals and non-target organisms [**24, 25**]. Safe ecological pesticides that do not leach residues into the environment have great importance [**26**].

Large quantities of synthetic pesticides are imported to Indi which is basically dependent on Agriculture as one of the main economic factors. These pesticides have a negative impact on the environment and human health. Neem biopesticides will be part of the appropriate solution for these environmental problems. This study is an attempt to generate prerequisite information on this valuable tree by quantifying the most active ingredient Azadirachtin and to relate it to the most effective ecological factors to generate elite trees for future plantations.

MATERIALS AND METHODS

Study area: Thirty six provenances of *Azadirachta indica* from all over India were planted in 1992 at Arid Forest Neem Research Field, Jodhpur. The details of these provenances such as provenance number, location, latitude, longitude and agro climatic zone given in table 1.

Seed collection: Three Seeds sample were collected randomly from each replications and the number of trees per provenances were twenty five.

Site characteristic: The studies were conducted at Arid Forest Research Institute, Jodhpur which is situated between 26° 45’ North latitude and 71° 03’ East longitudes, in the arid region of India. The description of the site is mentioned below.

Location: The provenances trial was established at AFRI, Neem field, Near Jhalamand Jodhpur in the state of Rajasthan, India, Latitude 26°40’N, Longitude 71° 15’E (Figure 1).

Neem seeds samples were collected directly from 36 seed sourced which covered ten agroclimatic zones of India. These regions cover the whole country. Seeds were collected from 10-20 different trees for each seed sources. Greenish yellow fruits harvested manually and were pooled together approx. 200

Table 1. Longitudinal, latitudinal positions and Agro climatic Zone of the regions from where the seeds of thirty six Neem provenances collected. The second column corresponds to the code assigned to the accession number

S.No.	Prov No.	Agroclimatic zone (AZ)	State	Location	Latitude	Longitude
1	P1	VIII	Rajasthan	Kota	25°10'N	75°52'E
2	P2	IX	Madhya Pradesh	Indore	22° 44'N	75°50'E
3	P3	IX	Madhya Pradesh	Ujjain	23°09'N	75°43'E
4	P4	XIV	Rajasthan	Jaisalmer	26°55'N	70°53'E
5	P5	XIV	Rajasthan	Bikanar	28°10'N	73°22'E
6	P6	XIV	Rajasthan	Jodhpur	26°40'N	71°15'E
7	P7	IX	Maharashtra	Pune	20°05'N	73°50'E
8	P8	IX	Maharashtra	Satra	19°40'N	74°12'E
9	P9	IX	Maharashtra	Solapur	17°42'N	76°20'E
10	P10	VIII	Rajasthan	Sawi madhopur	25°58'N	76°30'E
11	P11	IX	Maharashtra	Nagpur	21°10'N	79°20'E
12	P12	VIII	Uttar Pradesh	Jhansi	25°27'N	78°37'E
13	P13	V	Uttar Pradesh	Kanpur	26°28'N	80°24'E
14	P14	XIII	Gujarat	Rajkot	22°27'N	70°07'E
15	P15	XIII	Gujarat	Palampur	24°19'N	72°19'E
16	P16	XIII	Gujarat	Gandhinagar	23°15'N	72°17'E
17	P17	VIII	Rajasthan	Pali	26°55'N	70°53'E
18	P18	VI	Delhi	Delhi	23°38'N	77°12'E
19	P19	VI	Rajasthan	Sikar	26°55'N	75°52'E
20	P20	V	UttarPradesh	Mathura	27°28'N	77°41'E
21	P21	VI	Haryana	Gurgon	28°37'N	77°10'E
22	P22	IX	Maharashtra	Amarawati	20°45'N	78°50'E
23	P23	X	AndhraPradesh	Ravinagar	18°35'N	79°20'E
24	P24	VII	Bihar	Ranchi	23°23'N	85°23'E
25	P25	IV	Bihar	Muzapharpur	26°07'N	85°27'E
26	P26	X	Andhra Pradesh	Mulag	18°05'N	78°18'E
27	P27	III	West Benghal	Bankura	23°25'N	87°21'E
28	P28	VII	Madhya Pradesh	Raipur	21°15'N	81°41'E
29	P29	VII	Madhya Pradesh	Bilaspur	20°15'N	82°13'E
30	P30	VIII	Madhya Pradesh	Jabalpur	23°50'N	78°44'E
31	P31	VIII	Madhya Pradesh	Hoshangabad	20°50'N	78°40'E
32	P32	VIII	Madhya Pradesh	Shivpuri	25°40'N	77°44'E
33	P33	VIII	Madhya Pradesh	Maihar	23°34'N	80°55'E
34	P34	VIII	Madhya Pradesh	Suhagi	23°32'N	77°57'E
35	P35	VIII	Madhya Pradesh	Katni	23°47'N	80°27'E
36	P36	VIII	Madhya Pradesh	Riva	24°31'N	81°19'E

Table 2. Agro climatic zone of India from where seeds of different Neem provenances collected

Agro climatic Zone (AZ)	Description	Provenances
AZ- III	The lower Gangetic plain region	P27
AZ- IV	The middle Gangetic plain region	P25
AZ- V	The upper Gangetic plain region	P13,P20
AZ- VI	The trans – Gangetic plain region	P18,P19,P21
AZ- VII	The eastern plateau and hill region	P28,P29, P24,
AZ- VIII	The central plateau and hill region	P1,P10,P12,P17, P30,P30, P31,P32,P33,P34,P35,P36
AZ- IX	The western plateau and hill region	P2,P3,P7,P8,P9,P11,P22
AZ- X	The southern plateau and hill region	P23,P26
AZ- XIII	The Gujarat plain and hill region	P14,P15,P16
AZ- XIV	The western dry region	P4,P5,P6

gm. Seeds from each seed sources (Table 2). Homogenous lots of the seed were depulped, washed with water and dried under the shade. The dried seeds were stored at room temperature in air tight cotton bags.

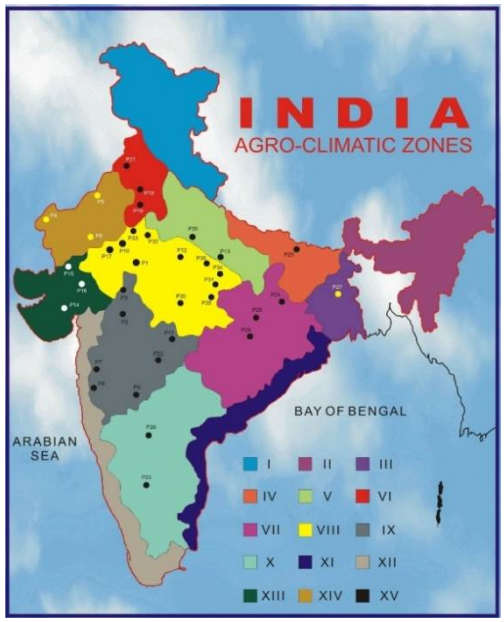


Figure 1. Neem Province from different Agro climatic Zones of India.

HPLC: Analytical High Performance Liquid Chromatography (Beckman HPLC, System Gold) (HPLC) separation was performed on ODS columns, ultra sphere C-18, 250×4.6 mm, programmed as follows: 40% acetonitrile in water (10 min) to 70% acetonitrile (10 min.) and finally to 100% acetonitrile (5 min.), all at 1 mL min^{-1}. The eluents were monitored at 214 nm. Quantification of the azadirachtin was based on injections of known quantities of standard samples. The ultra-violet spectra of crude substances were determined using an on-line diode array detector on a running on acetonitrile/water solvent system. Each sample was analyzed three times.

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 Provence tril. Analysis of variance was carried out following the procedure given by Panse and Sukhatme [27].

RESULTS AND DISCUSSION

The samples were analysed for the azadirachtin content in NSK using the methods mentioned above and the result were presented in table 3. The concentration of Azadirachtin varies from 0.11% to 0.93% by weight of NSK in different seed sources of India. The maximum mean of azadirachtin percentage was investigated in Palanpur provenance (0.93%) which was at par with Shivpuri provenance (0.92%) and Mulag provenance (0.92%).The lowest mean of azadirachtin percentage was analysed in Bikanar provenance (0.11%), which was at par with Bilaspur provenance (0.14 %) as shown in (Table 3).

One-way analysis of variance was conducted to see the significance difference in Azadirachtin and percentage in Agro climatic zone. Results indicate that there were very highly significant differences ($p \leq 0.001$) in Azadirachtin % in agro climatic zone (Table 4). The maximum mean of azadirachtin percentage were analysed in AZ XIII (0.80%), which was at par with AZ III (0.80%), AZ IV (0.63%) and AZ X (0.58%). The lowest mean of azadirachtin were found in AZ XIV (0.20 %)

which was at par with AZ VII (0.25%), AZ VI (0.28%), AZ (0.32%) and AZ VIII (0.41).Thus, the present investigation indicates that azadirachtin content vary in different seed sources.

Table 3. Azadirachtin percentage in different provenances of *A. indica*

Prov. No.	No. of sample	Aza (%) Original Mean± SE	95% Confidence Interval for mean of Aza. %		Prov. No.	No. of sample	Aza (%) Original Mean± SE	95% Confidence Interval for mean of Aza. %	
			Lower Bound	Upper Bound				Lower Bound	Upper Bound
P1	3	0.27± 0.010	37.08	39.00	P19	3	0.31±0.018	33.63	44.00
P2	3	0.30±0.006	36.38	44.35	P20	3	0.32±0.010	37.84	43.01
P3	3	0.30±0.000	46.19	47.92	P21	3	0.16±0.006	38.66	42.86
P4	3	0.20±0.003	39.16	40.65	P22	3	0.82±0.016	39.49	43.87
P5	3	0.11±0.003	35.64	41.75	P23	3	0.24±0.013	34.48	41.78
P6	3	0.30±0.006	42.72	52.96	P24	3	0.39±0.003	34.89	39.25
P7	3	0.66±0.028	41.68	49.39	P25	3	0.63±0.016	42.43	55.90
P8	3	0.67±0.012	46.27	52.69	P26	3	0.92±0.033	47.00	51.35
P9	3	0.16±0.014	36.78	47.61	P27	3	0.80±0.000	40.42	43.53
P10	3	0.22±0.020	36.61	40.17	P28	3	0.21±0.010	40.31	42.18
P11	3	0.30±0.006	42.73	51.53	P29	3	0.14±0.003	38.32	41.56
P12	3	0.52±0.010	37.98	40.63	P30	3	0.66±0.003	48.56	49.45
P13	3	0.32±0.015	39.12	46.52	P31	3	0.20±0.008	35.85	45.06
P14	3	0.66±0.010	38.51	44.20	P32	3	0.92±0.016	47.09	51.27
P15	3	0.93±0.016	47.33	52.66	P33	3	0.27±0.023	43.45	50.30
P16	3	0.82±0.016	40.99	55.48	P34	3	0.41±0.006	38.42	45.85
P17	3	0.66±0.010	36.35	48.48	P35	3	0.33±0.010	42.44	54.29
P18	3	0.37±0.020	37.02	45.46	P36	3	0.43±0.010	38.71	50.47

Azadirachtin%: F $_{\mathbf{cal}(35, 72)}$ Provenances=327.037****** = p ≤.001.
(Very highly significant)

Table 4. Azadirachtin percent in different provenances of *A. indica* in agro climatic zone

Agro climatic zone(AZ)	Mean Azadirachtin % Original ±SE
III	0.80^{d}±0.000
IV	0.63^{cd} ±0.016
V	0.32^{ab}±0.008
VI	0.28^{ab}±0.031
VII	0.25^{ab}±0.037
VIII	0.41^{abc}±0.036
IX	0.46^{bc}±0.052
X	0.58^{cd} ±0.151
XIII	0.80^{d} ±0.040
XIV	0.20^{a}±0.026

Azadirachtin %: F cal $_{(9, 98)}$ Agro climatic zone = 9.009***
*** = p≤ .001. (Very highly significant)
Any two means having a common superscript in the column for Azadirachtin % are not significantly different at 5% level as separated by Duncan Multiple Range Test (DMRT).

The results showed great variations in the active ingredient, AZ content, between the different seeds, which were collected from different ecological and geographical zones. Similar results were reported by other investigators [**20, 21**]. Ermel (1995) investigated different samples from different parts of the world and showed great variations between and within countries [**28**]. These variations could be attributed to the edaphic and climatic factors, which vary in India from the desert in the upper north and from hilly areas in the Western to open plains in the Central and Eastern parts. These results were also in line with other detailed investigations of Gruber [**29**] on AZ-content of 47 marked trees from five locations in Nicaragua over a period of 4 years. This study revealed a great influence of edaphic climatic factors on the synthesis and degradation of AZ. Kaushik *et al.*, revealed the

concentration of azadirachtin varied from 200 to 16,000 ppm and azadirachtin content was found to be affected by climate and habitat [30]. Annual variation in azadirachtin content was significant.

There is increasing demand for quality planting material for neem plantations. However, individual neem trees vary in their chemical make-up as the oil-content and limonoids-content of neem trees are governed by genetic and environmental factors. Efforts are lacking for the selection of neem trees based on AZ-content and the oil-content. The present study provides some of the needed information for such selections for the new plantations.

APPLICATION

The selection of superior plus trees for industrial plantations would help to improve the overall productivity of neem in terms seed, oil and azadirachtin.

CONCLUSION

There is increasing demand for quality planting material for neem plantations. However, individual neem trees vary in their chemical make-up as the oil-content and limonoids-content of neem trees are governed by genetic and environmental factors. Efforts are lacking for the selection of neem trees based on Az-content and the oil-content. The present study provides some of the needed information for such selections for the new plantations.

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