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# Variability in Neem (*Azadirachta indica)* with Respect to Azadirachtin Content

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There is a controversy over variations in azadirachtin content in neem (*Azadirachta indica*) seeds among various provenances and countries. Also, variations in azadirachtins are usually attributed to climatic conditions such as temperature and humidity. The present study was undertaken to evaluate qualitative and quantitative variability in azadirachtins A and B among various neem provenances or individual neem trees. Forty-three provenances of India were examined for intraprovenance variability in azadirachtin A and B content and oil percentage. Twenty-eight individual neem trees from five provenances of different agroclimatic regions were also examined for interprovenance variability. The azadirachtins were quantified using reversed phase analytical HPLC. There were wide variations in oil and azadirachtin contents among different provenances. Azadirachtin A ranged from 556.9 to 3030.8 mg $kg^{-1}$ of kernels, whereas azadirachtin B was in the range 43.1−590.6 mg $kg^{-1}$ of kernel among the provenances investigated. Analysis of variance among various neem provenances showed significant differences in oil content, azadirachtin A, total azadirachtin (A + B), and A:B ratio. There were individuals with high and low azadirachtins within a single provenance, and this trend was observed in all of the provenances selected from five agroclimatic regions of the country. Variations among individual trees of a particular provenance indicated that climatic factors such as rainfall, humidity, or temperature did not influence azadirachtin content in the neem trees. The present study shows that there are individual genetic differences among neem trees. A systematic study for tree improvement with a population of mother trees with desired traits should be undertaken by performing half-sib progeny trials and further selections by clonal propagations. The role of genetic makeup needs further research.

**KEYWORDS: Neem; azadirachtin A; azadirachtin B; oil content; variability; provenances; climatic factors; genetic makeup**

## INTRODUCTION

*Azadirachta indica* A. Juss. (neem) of the family Meliaceae is native to the Indian subcontinent. Neem, today, is grown in many Asian countries and in tropical regions of the western hemisphere. Leaves and seeds of this tree have traditionally been used for centuries for treatment of human ailments and control of pests (*1*). Azadirachtin ($C_{35}H_{44}O_{16}$), a tetranortriterpenoid from the neem kernel, has been rated as the most potent naturally occurring insect feeding deterrent (*2, 3*) and has generated wide academic and industrial interests (*4*−*6*). Azadirachtins function as natural insect control agents because of their antifeedant as well as insect growth regulatory properties (*7, 8*).

Azadirachtin A is the principal compound for which extensive structural and biological studies have been carried out (*9*−*11*). Rembold (*11*) isolated six related compounds (azadirachtins B−G), whereas Govindachari et al. (*12*) reported seven compounds (azadirachtins A, B, D, F, H, I, and K) of closely related structures isolated from neem kernels. Of nine isomers of azadirachtin reported in the literature, azadirachtins A and B, the major active metabolites from neem seeds, are considered cardinal to the commercialization of neem for biopesticides. Klenk et al. (*13*) and Rembold (*14*) have reported that azadirachtin B occurs to the extent of one part to five parts of azadirachtin A, whereas others (azadirachtins C−G) are only one part each to hundred parts of A.

There is often a mention in the literature about variations of azadirachtin content in neem from different countries or different regions within a country. Extensive variation in the azadirachtin content of neem ecotypes has been reported by Kumar and Parmar (*15*) and others; however, high variability could not be attributed to any specific environmental, edaphic, or genetic conditions. Ermel (*16*) and Venkateswarlu et al. (*17*) attributed these variations in azadirachtin content to local environmental conditions such as humidity, rainfall, temperature, or season. Sidhu and Behl (*18*) in their study of seasonal (monsoon and winter) variations in azadirachtin A, B, and F in seeds of *A. indica* have reported that winter stress appears to favor the synthesis of azadirachtins B and F in neem seeds.

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**Figure 1.** Map of India showing neem seed collection sites.

If the hypothesis that environmental conditions such as temperature, humidity, and moisture stress or its abundance would influence azadirachtin quality and quantity is accepted, then all of the individuals of a particular locality may have nearly uniform azadirachtin content. Although there could be variations due to climate × ecotype interactions resulting in within-site variability in azadirachtin content, it should be possible to identify good provenances and ecotypes and easy to select ideal ecologically defined sites for growing neem and predicting high azadirachtin output; however, no such conclusive information is available in the literature. In view of the lack of any systematic study on the qualitative and quantitative variability in azadirachtins A and B among various neem provenances or individual neem trees, the present study was undertaken. The objective of the study was to evaluate large populations from different agroclimatic zones. Several individual trees within a provenance were also examined for variability in azadirachtin content.

## MATERIALS AND METHODS

Neem seed samples were collected from healthy mature trees from 43 provenances of seven states of India (**Figure 1**), namely, Bihar (Barauni, Bhagalpur, Chapra, Darbhanga, Hazipur, Muzzafarpur, Patna, Samastipur, and Siwan), Jharkhand (Ranchi), Haryana (Rohtak), Rajasthan (Ajmer, Jaipur, Jaisalmer, Jodhpur, and Sri Ganganagar), Tamil Nadu (Kanyakumari), Uttranchal (Dehradun and Haridwar), and Uttar Pradesh (Agra, Auraiya, Badaun, Bijnor, Bulandshahar, Farrukhabad, Fatehpur, Gazipur, Hathras, JP Nagar, Kanpur, Kannauj, Kheri, Lucknow, Mathura, Mau, Meerut, Moradabad, Muzzafarnagar, Orai, Pratapgarh, Rampur, Shahjahanpur, and Varanasi) during 1999−2001. Each provenance contained a mixed seed lot from 20−25 trees of a comparable age group. Seed samples collected from randomly selected individual trees from five sites (Agra, Auraiya, Dehradun, JP Nagar, and Kanyakumari), each in a different agroclimatic zone, were analyzed for oil and azadirachtin (A, B) content. [Samples of collections were submitted to the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, for Indigenous Collection (IC) numbers and cryopreservation. Results of only those provenances and individuals are reported where IC numbers have been obtained.]

One kilogram of fruit was hand-collected from branches of trees when the seeds became greenish-yellow to yellowish ripening stage. Collected seeds were depulped under running tap water and shade-dried for 7 days. Seeds were measured for phenological traits, particularly seed area. Seeds were then decorticated, and kernels were processed for oil and azadirachtin analysis. One hundred grams of neem kernels was defatted three times with hexane using a commercial blender at 35 °C for 15 min. The residue thus obtained was extracted with 90% ethanol in a manner similar to that for hexane extraction. The enriched azadirachtin content from the ethanolic extract was isolated following the method of Sidhu and Behl (*18*).

**Quantitative Analysis.** Analyses were performed on a Waters liquid chromatograph equipped with a Waters automated gradient controller, a Waters 501 solvent delivery system, a Rheodyne 7125 sample injector fitted with a 20 $\mu$L loop, and a Waters 484 variable absorbance detector, with Waters Millenium$^{32}$ software. A Waters Spherisorb C8 (4.6 × 250 mm) analytical column equipped with a Waters Spherisorb S5 C8 guard cartridge (4.6 × 10 mm) was used. Azadirachtins were detected at 220 nm. A KT-25S degassing device (Shodex degasser, Tokyo, Japan) was used to degas the solvents. The mobile phase consisted of an isocratic mixture of acetonitrile/methanol/water (23:22:55) at a flow rate of 1.0 mL min$^{-1}$. The results are presented on a percent weight basis, and the same were quantified using external standards of azadirachtins A and B obtained from Trifolio-M GmbH, Lahnau, Germany.

## RESULTS

Forty-three provenances of neem were screened for their azadirachtin A and B contents. Azadirachtin A and B values (average of three replicates) are given on kernel dry weight basis and arranged in ascending order of azadirachtin A concentration. Seed area, azadirachtins A and B, total (A + B), ratio of azadirachtin A to B, and oil content of the investigated provenances are also presented in **Table 1**.

**Quantitative Determination of Azadirachtins.** Chromatographic separation of azadirachtins A and B was improved from the earlier reported studies. An isocratic mixture of acetonitrile/methanol/water (23:25:55) at a flow rate of 1.0 mL min$^{-1}$ on a Waters Spherisorb C8 (4.6 × 250 mm) column gave better resolution of azadirachtins A and B than that of aqueous acetonitrile. Azadirachtin A appeared at 19.2 min, whereas the $t_R$ of azadirachtin B was 20.2 min, with capacity factors ($k'$) of 6.31 and 6.71, respectively. A relative retention ($\alpha$ value) of 1.06 indicates good separation of two peaks (**Table 2**). When azadirachtin values among trees or provenances are compared, it is essential to specify if the estimations are for total azadirachtin (A + B) or only for azadirachtin A. Lack of clarification often leads to misleading comparisons between various studies.

**Interprovenance Variability.** Seed area, percentage of oil, azadirachtin content of A, B, A + B, and their ratio in 43 provenances are shown in **Table 1**. Seed area ranged from 68.8 mm$^2$ in Jodhpur to 112.3 mm$^2$ in Farrukhabad provenance (both of Uttar Pradesh), whereas average seed area among all of the provenances was 90 mm$^2$. This trait was not found to be significantly related to other parameters taken in this study. Oil content ranged from 32.9 to 51.6% (with an average of 41.5%), the lowest being in Jodhpur (Rajasthan) and the highest in Fatehpur. It was significant to note that oil percentage in Rajasthan provenances that fall under semiarid to highly arid regions such as Jodhpur (32.9%), Jaipur (33.5%), Jaisalmer (34.5%), and Sri Ganganagar (37.26%) was relatively very low. Kanyakumari in Tamil Nadu also had a low oil percentage (35.05%); this region is arid, being at the tip of the ocean and under a constant stress of salinity. There were statistically significant differences in oil content among various (43) provenances ($P < 0.001$) with an $F$ value of 9.2 (**Table 3**).

**Table 1.** Seed Area, Oil Content, and Azadirachtins (A and B) among Neem Accessions from Different Agroclimatic Zones of India

| | provenance | seed area ($mm^2$) | aza A (mg/kg of kernel wt) | aza B (mg/kg of kernel w). | total A + B | A:B ratio | oil (%) |
|---|---|---|---|---|---|---|---|
| 1 | Orai[a] | 83.5 | 556.9 | 225.7 | 782.6 | 2.47 | 44.7 |
| 2 | Chapra[b] | 98.1 | 678.6 | 111.5 | 790.2 | 6.13 | 39.7 |
| 3 | Auraiya[a] | 83.7 | 804.2 | 68.1 | 872.3 | 33.07 | 37.1 |
| 4 | Kanyakumari[c] | 88.9 | 829.9 | 379.4 | 1209.3 | 2.95 | 35.0 |
| 5 | Bijnor[a] | 73.4 | 834.8 | 177.1 | 1011.9 | 4.71 | 46.2 |
| 6 | Kannauj[a] | 102.1 | 880.2 | 312.3 | 1192.5 | 3.32 | 36.4 |
| 7 | Rampur[a] | 72.3 | 929.7 | 88.9 | 1018.7 | 10.48 | 37.3 |
| 8 | Gazipur[a] | 98.8 | 942.6 | 66.2 | 1008.9 | 14.31 | 42.7 |
| 9 | Ajmer[d] | 86.3 | 955.5 | 316.2 | 1271.7 | 3.02 | 33.2 |
| 10 | Bhagalpur[b] | 100.3 | 980.6 | 101.9 | 1082.5 | 9.62 | 43.6 |
| 11 | Barauni[b] | 90.9 | 982.7 | 147.4 | 1130.1 | 7.25 | 41.8 |
| 12 | Darbhanga[b] | 89.9 | 1002.5 | 119.7 | 1122.1 | 8.42 | 45.5 |
| 13 | Jodhpur[d] | 68.8 | 1003.1 | 103.2 | 1106.3 | 9.71 | 32.9 |
| 14 | Moradabad[a] | 101.3 | 1013.3 | 214.7 | 1228.1 | 6.41 | 50.1 |
| 15 | Farrukhabad[a] | 112.3 | 1022.5 | 94.0 | 1116.5 | 10.87 | 42.5 |
| 16 | Mathura[a] | 90.5 | 1071.8 | 58.7 | 1130.5 | 31.41 | 45.3 |
| 17 | Kheri[a] | 91.6 | 1082.6 | 173.2 | 1255.7 | 5.88 | 43.1 |
| 18 | Jaisalmer[d] | 75.2 | 1092.2 | 426.2 | 1518.4 | 2.56 | 34.5 |
| 19 | Hathras[a] | 82.4 | 1126.9 | 134.4 | 1261.2 | 8.40 | 42.4 |
| 20 | Ranchi[g] | 92.5 | 1159.8 | 190.1 | 1349.9 | 6.09 | 41.2 |
| 21 | Muzzafarpur[b] | 90.1 | 1169.4 | 141.5 | 1310.9 | 8.26 | 41.6 |
| 22 | Bulandshahar[a] | 97.0 | 1205.1 | 139.4 | 1344.5 | 9.79 | 49.7 |
| 23 | Haridwar[e] | 83.3 | 1231.0 | 182.8 | 1413.8 | 7.17 | 46.1 |
| 24 | Hazipur[b] | 101.1 | 1254.4 | 96.6 | 1351.0 | 13.04 | 42.9 |
| 25 | Patna[b] | 86.2 | 1272.6 | 228.3 | 1500.9 | 5.58 | 41.1 |
| 26 | Lucknow[a] | 105.2 | 1334.2 | 139.4 | 1473.6 | 9.66 | 45.2 |
| 27 | Badaun[a] | 97.8 | 1388.1 | 143.9 | 1532.0 | 9.64 | 35.2 |
| 28 | Dehradun[e] | 89.3 | 1459.7 | 326.6 | 1786.3 | 4.72 | 45.3 |
| 29 | JP Nagar[a] | 74.9 | 1505.2 | 590.6 | 2095.9 | 6.03 | 37.1 |
| 30 | Varanasi[a] | 104.8 | 1531.7 | 69.7 | 1601.5 | 24.54 | 48.3 |
| 31 | Muzzafarnagar[a] | 88.8 | 1534.5 | 149.7 | 1684.2 | 10.27 | 40.1 |
| 32 | Meerut[a] | 101.2 | 1573.8 | 105.2 | 1679.0 | 22.63 | 40.8 |
| 33 | Agra[a] | 76.2 | 1576.2 | 261.9 | 1838.0 | 8.16 | 43.9 |
| 34 | Siwan[b] | 81.1 | 1629.0 | 96.0 | 1725.0 | 17.0 | 42.8 |
| 35 | Kanpur[a] | 80.5 | 1671.4 | 68.9 | 1740.4 | 23.14 | 40.8 |
| 36 | Pratapgarh[a] | 78.9 | 1705.9 | 100.8 | 1806.7 | 17.12 | 38.6 |
| 37 | Jaipur[d] | 92.6 | 1706.4 | 251.5 | 1957.9 | 6.78 | 33.5 |
| 38 | Samastipur[b] | 84.5 | 1916.5 | 95.0 | 2011.5 | 20.19 | 45.0 |
| 39 | Fatehpur[a] | 97.0 | 1941.2 | 43.1 | 1984.3 | 98.15 | 51.6 |
| 40 | Rohtak[f] | 86.6 | 2032.9 | 243.9 | 2276.8 | 8.33 | 37.9 |
| 41 | Mau[a] | 92.6 | 2058.2 | 100.3 | 2158.5 | 20.53 | 43.2 |
| 42 | Sri Ganganagar[d] | 89.0 | 2400.0 | 172.3 | 2572.3 | 13.92 | 37.3 |
| 43 | Shahjahanpur[a] | 110.9 | 3030.8 | 134.1 | 3165.0 | 24.32 | 40.6 |
| | CD at 1% | 61.10 | 1023.04 | 326.10 | 1126.3 | 33.87 | 4.45 |
| | CD at 5% | 45.94 | 769.20 | 245.19 | 846.8 | 25.47 | 3.34 |

[a] Uttar Pradesh. [b] Bihar. [c] Tamil Nadu. [d] Rajasthan. [e] Uttranchal. [f] Haryana. [g] Jharkhand.

**Table 2.** Retention Times ($t_R$), Capacity Factors ($k'$), and Relative Retentions ($\alpha$) of Azadirachtins A and B from Neem Kernels

| azadirachtin | $t_R$ (min) | $k'$ | $\alpha$ |
|---|---|---|---|
| A | 19.16 | 6.31 | 1.06 |
| B | 20.20 | 6.71 | |

**Azadirachtins A and B.** Azadirachtins A and B varied greatly among the 43 provenances investigated. Azadirachtin A ranged from 556.9 to 3030.8 mg $kg^{-1}$ of kernel, the lowest being in Orai and the highest in Shahjahanpur with an average of 1327.4 mg $kg^{-1}$ of kernel. Among the eight high azadirachtin yielding provenances, four fell in Uttar Pradesh, two in Rajasthan, and one each in Bihar and Haryana, suggesting no definite pattern. Differences in azadirachtin A among various provenances were found to be statistically significant ($P < 0.01$) with an $F$ value of 2.89 (**Table 3**). Some of the provenances of arid regions (Ajmer, Jodhpur, and Kanyakumari) that were found to be low in oil percentage had relatively low azadirachtin content.

**Table 3.** Analysis of Variance between Seed Area, Oil Content, Azadirachtins A and B, A + B, and A:B Ratio among Different Provenances[a]

| source | degree of freedom | SS | MS | $F$ value |
|---|---|---|---|---|
| seed area ($mm^2$) | 42 | 11446.1 | 317.9 | $0.4^{NS}$ |
| oil (%) | 42 | 1393.97 | 38.7 | 9.2** |
| azadirachtin A | 42 | $2.31 \times 10^7$ | 642225.1 | 2.9* |
| azadirachtin B | 42 | 1168336 | 32453.7 | $1.4^{NS}$ |
| total azadirachtin (A + B) | 42 | $2.32 \times 10^7$ | 647116.4 | 2.4* |
| A:B ratio | 42 | 28426.2 | 789.6 | 3.2* |

[a] Results are of two-way ANOVA [seed parameters, oil content, azadirachtin A, azadirachtin B, total azadirachtin (A + B), A:B ratio, and various provenances]; total = 36; * = $P < 0.01$; ** = $P < 0.001$; NS = not significant.

Azadirachtin B had a very wide range from a low of 43.1 to 590.6 mg $kg^{-1}$, the lowest being in Fatehpur and the highest in JP Nagar with an average of 171.9 mg $kg^{-1}$. Azadirachtin A dominated over B in all of the provenances investigated; however, the ratio of azadirachtin A:B varied greatly among

**Table 4.** Correlation Coefficient (*R* Values) among Seed Area, Oil Content, Azadirachtins A and B, A + B, and A:B Ratio[a]

| source | seed area ($mm^2$) | oil (%) | aza A | aza B | aza A + B | A:B ratio |
|---|---|---|---|---|---|---|
| seed area ($mm^2$) | 1.00 | | | | | |
| oil (%) | $0.09^{NS}$ | 1.0 | | | | |
| aza A | $0.04^{NS}$ | $0.09^{NS}$ | 1.0 | | | |
| aza B | $-0.02^{NS}$ | $-0.11^{NS}$ | $0.04^{NS}$ | 1.0 | | |
| total (A + B) | $0.04^{NS}$ | $0.06^{NS}$ | 0.96** | 0.30** | 1.0 | |
| A:B ratio | $0.08^{NS}$ | $0.24^{NS}$ | 0.45** | $-0.30^{NS}$ | 0.36* | 1.0 |

[a] * = $P < 0.01$; ** = $P < 0.001$; NS = not significant.

the provenances. It was in the range of 2.5−98.1 with an average of 13.4, the lowest being in Orai and the highest in Fatehpur provenance. Most of the provenances with low oil content had a low ratio of azadirachtins.

It is customary to report total azadirachtins. Total azadirachtin (A + B) content varied from 782.6 to 3164.9 mg $kg^{-1}$ with an average of 1499.3 mg $kg^{-1}$. Analysis of variance for seed area, oil content, and azadirachtin content and ratio among various neem provenances showed significant differences in oil content, azadirachtin A, total azadirachtin (A + B), and A:B ratio (**Table 3**). Correlation coefficients among the parameters analyzed by Pearson matrix showed significant correlation between azadirachtin A, A + B, and A:B among the parameters analyzed (**Table 4**).

**Individual Tree Variations.** Twenty-eight individual trees from five different agroclimatic zones were screened for variability in oil and azadirachtin content among trees of a particular site. Data from individual trees of each zone have been presented in **Table 5**. Azadirachtin varied considerably between individual trees of a particular agroclimatic zone. Azadirachtin A ranged from 68.5 to 2926 mg $kg^{-1}$ in the population of 28 trees of five agroclimatic regions, whereas azadirachtin B ranged from 18.3 to 1553.0 with an average of 239.9 mg $kg^{-1}$.

The ratio of azadirachtins (A:B) varied widely (1.2−133.8) at the JP Nagar site, whereas it fell in a relatively narrow range (1.2−3.8) at Kanyakumari. An individual tree (IC268650) at JP Nagar had a very high azadirachtin B content (1553 mg $kg^{-1}$) with a nearly equal amount of azadirachtin A (1956.5 mg $kg^{-1}$. However, another tree (IC268652) from the same zone contained 2547.6 mg $kg^{-1}$ azadirachtin A and only a negligible amount of azadirachtin B (19.1 mg $kg^{-1}$) with an A:B ratio of 133.78. Such trees may be distinct in their extreme ranges, but almost all of the trees varied in the proportion of these two major metabolites. Kanyakumari had a distinctness of relatively uniform trees with respect to the ratio of azadirachtin A to B. It ranged from 1.2 to 3.8 with an average of 2.55.

## DISCUSSION

This study of 43 provenances of neem and 28 trees from five agroclimatic zones of India provides answers to the old controversy related to variability in azadirachtins (A and B) among neem trees. A large variability was observed in azadirachtin content. Azadirachtin A varied from 556.9 to 3030.8 mg $kg^{-1}$ with an average of 1327.4 mg $kg^{-1}$ among the 43 provenances. Certain individual trees (IC268646) within a provenance (JP Nagar, Uttar Pradesh) had a negligible amount of 68.5 mg $kg^{-1}$ of azadirachtin A. Even within a provenance there was a great variability in azadirachtin A content. JP Nagar provenance was a unique case study where azadirachtin A content varied from 68.5 to 2558.5 mg $kg^{-1}$, although the majority of the trees had 1265−2558 mg $kg^{-1}$. Similar variations

**Table 5.** Variation in Azadirachtins A, B, A + B, and A:B Ratio between Individual Trees of Five Agroclimatic Zones

| agroclimatic zone[a] | Indigenous Collection (IC) no. | aza A (mg/kg of kernel wt) | aza B (mg/kg of kernel wt) | aza A + B | aza A:B ratio |
|---|---|---|---|---|---|
| Agra | 268561 | 748.8 ± 15.3 | 80.5 ± 0.8 | 829.2 ± 15.1 | 9.3 ± 0.2 |
| | 268562 | 1003.2 ± 6.0 | 91.6 ± 1.8 | 11094.8 ± 4.5 | 10.9 ± 0.3 |
| | 268563 | 1033.0 ± 13.5 | 191.5 ± 1.1 | 1224.5 ± 14.0 | 5.4 ± 0.06 |
| | 268564 | 1171.1 ± 13.5 | 33.2 ± 2.2 | 1204.2 ± 11.4 | 35.4 ± 2.6 |
| | 268565 | 1215.7 ± 8.9 | 151.3 ± 2.2 | 1367.0 ± 9.1 | 8.0 ± 0.1 |
| | 268566 | 1231.1 ± 5.3 | 142.7 ± 1.4 | 1373.8 ± 5.3 | 8.6 ± 0.1 |
| | 268567 | 2926.4 ± 39.7 | 427.0 ± 5.3 | 3353.4 ± 41.1 | 6.8 ± 0.1 |
| Auraiya | 268595 | 507.0 ± 5.7 | 161.9 ± 1.5 | 669.0 ± 4.3 | 3.1 ± 0.1 |
| | 268596 | 519.7 ± 1.2 | 18.3 ± 0.5 | 538.1 ± 1.6 | 28.4 ± 0.7 |
| | 268597 | 735.9 ± 16.9 | 41.3 ± 0.7 | 777.2 ± 17.3 | 17.8 ± 0.3 |
| | 268598 | 1174.0 ± 7.0 | 21.5 ± 1.3 | 1195.4 ± 5.7 | 54.8 ± 3.7 |
| | 268599 | 1624.0 ± 10.1 | 135.5 ± 1.2 | 1759.5 ± 9.2 | 12.0 ± 0.2 |
| Dehradun | 268688 | 663.3 ± 2.3 | 382.2 ± 1.6 | 1045.5 ± 3.3 | 1.7 ± 0.1 |
| | 268689 | 1211.1 ± 16.2 | 193.5 ± 1.1 | 1404.6 ± 17.1 | 6.2 ± 0.05 |
| | 268690 | 2499.1 ± 11.0 | 404.6 ± 8.0 | 2903.7 ± 7.2 | 6.2 ± 0.1 |
| JP Nagar | 268646 | 68.5 ± 0.5 | 61.1 ± 0.9 | 129.6 ± 0.4 | 1.1 ± 0.02 |
| | 268647 | 972.7 ± 15.0 | 93.8 ± 1.2 | 1066.5 ± 16.2 | 10.4 ± 0.04 |
| | 268648 | 1265.5 ± 12.8 | 106.1 ± 4.2 | 1371.6 ± 16.0 | 11.9 ± 0.4 |
| | 268649 | 1655.3 ± 6.1 | 291.5 ± 0.7 | 1946.8 ± 6.3 | 5.7 ± 0.02 |
| | 268650 | 1956.5 ± 6.9 | 1553.0 ± 8.9 | 3509.5 ± 2.6 | 1.2 ± 0.02 |
| | 268651 | 2496.1 ± 10.2 | 158.5 ± 1.4 | 2654.6 ± 9.1 | 15.7 ± 0.2 |
| | 268652 | 2547.6 ± 14.4 | 19.1 ± 0.9 | 2566.7 ± 15.3 | 133.8 ± 6.0 |
| | 268653 | 2558.5 ± 33.3 | 54.3 ± 0.6 | 2612.8 ± 33.8 | 47.1 ± 0.2 |
| Kanyakumari | 268605 | 757.5 ± 9.1 | 285.1 ± 3.8 | 1042.5 ± 12.7 | 2.6 ± 0.01 |
| | 268606 | 766.5 ± 11.5 | 622.4 ± 3.8 | 1388.9 ± 8.8 | 1.2 ± 0.02 |
| | 268607 | 848.5 ± 5.1 | 364.5 ± 9.6 | 1213.0 ± 5.9 | 2.3 ± 0.1 |
| | 268608 | 963.3 ± 11.4 | 252.8 ± 10.1 | 1216.1 ± 16.9 | 3.8 ± 0.1 |
| | 268609 | 1053.7 ± 14.6 | 381.1 ± 0.9 | 1434.8 ± 13.9 | 2.8 ± 0.04 |

[a] Agra, Auraiya and JP Nagar, Uttar Pradesh; Dehradun, Uttranchal; Kanyakumari, Tamil Nadu.

were observed in azadirachtin B, although no correlation between azadirachtins A and B could be established.

Klenk et al. (*13*) and Rembold (*14*) have reported that of nine isomers of azadirachtins known from the seed extracts, azadirachtin B occurred to the extent of one part to five parts of A, whereas the others (C−G) are one part each to one hundred parts of azadirachtin A. Govindachari et al. (*19*) isolated azadirachtins by preparative HPLC and found 980 mg $kg^{-1}$ of azadirachtin A and 336 mg $kg^{-1}$ of azadirachtin B in neem kernel. We have observed that the ratio of A to B may be from as low as 1.12 to a high of 98.1. The average ratio among 43 provenances was 13.4, whereas it was 16.2 in 28 individuals of five provenances. The study suggests that azadirachtin A is usually 13−16 times higher than azadirachtin B, but wide individual differences can be observed among a natural population. One may have to select trees tailored to the requirements.

On a total azadirachtin (A + B) basis, the neem seeds were found to have 782.6−3164.9 mg $kg^{-1}$ with an average of 1499.3 mg $kg^{-1}$, although some individual trees (IC268567, Agra) with 3353.4 and (IC268650, JP Nagar) 3509.5 mg $kg^{-1}$ of azadirachtins were also observed. Schroeder and Nakanishi (*20*), who isolated azadirachtins by flash chromatography, reported >0.25% of azadirachtin content in neem seeds. Yamasaki et al. (*21*) also isolated azadirachtins by flash chromatography and reported 56 mg $kg^{-1}$ (99% pure) azadirachtin from neem seeds. Jarvis et al. (*22*) have isolated triterpenoids from neem seeds by supercritical fluid chromatography and reported <0.3% of 11 tetranortriterpenoids including azadirachtins.

Ermel et al. (*23*) determined the azadirachtin content of neem seed kernels from various geographic regions of the world and reported great variability among samples from different countries. Kumar and Parmar (*15*) also investigated azadirachtin content from some neem ecotypes of India and reported the causes for variability in azadirachtin content as high rainfall, extreme drought, and high humidity.

Average of maximum and minimum temperatures, relative humidity, and rainfall at different provenances were monitored throughout the year during the present study. Some regions are distinct in their climatic traits. The range of highs at Kanyakumari (Tamil Nadu) was between 28 and to 36 °C throughout the year, whereas Agra crossed 43 °C during the summer months and had a minimum of 4.7 °C during January. The range of low temperature at Kanyakumari was 18.75−31.4 °C throughout the whole year. Jaisalmer, which falls in the extremely arid zone of the country, had a maximum temperature ranging between 39 and 48.5 °C. Relative humidity during the summer months was <20% in extremely arid regions, 30−40% in most of the indogangetic plains, and nearly 50% in Dehradun. It was >60% throughout the year in Kanyakumari. Highest rainfall was observed in Dehradun during the rainy months, whereas it was lowest in Kanyakumari during the same period. Average rainfall varied considerably among the zones investigated.

Most of the cases with low oil percentage were observed from regions under stress of aridity, salinity, or alkalinity. Jodhpur, Jaisalmer, and Ajmer in the dry zone with low humidity and high summer temperature and Kanyakumari with a stress of salinity had <35% oil. These areas also had a low ratio of azadirachtin A to B. Jaisalmer, for example, is an extremely arid site. It had 34.5% oil content, and the azadirachtin A:B ratio was 2.56. Kanyakumari, located at the southern tip of the landmass, was in a similar range. These are only trends because there were exceptions, and no significant correlation between oil and azadirachtin content was observed (**Table 4**). Kanyakumari, which had the least variability in temperature and humidity throughout the year, also had the least variability in azadirachtin A and B contents and their ratios. No such conclusion could be drawn for other areas where there is a large variability in daily and seasonal climatic conditions.

It is crucial to study a wide range of samples before concluding that there are variations among countries, provenances, or even a large population. It is also essential to compare results of different extraction methods from the uniform seed lot. Results of extraction techniques using different seed lots can lead to misleading information as there is a wide variability among neem trees and populations. The conclusion that the potential for azadirachtin A and B accumulation was influenced by environmental conditions could not be drawn because individual trees of a particular agroclimatic zone showed different trends.

Hasty conclusions of proposing a particular country or provenance yielding high azadirachtin may be risky. Findings based on small sample size or conclusions based on a multitude of factors can be misleading. With nearly 70 countries in the world interested in raising neem plantations, it is crucial that issues regarding variability in neem are understood. It can be concluded from the present study that there are individual genetic differences among neem trees and that synthesis of azadirachtins is not dependent upon temperature, humidity, or rainfall.

Genetic variability of azadirachtin may affect the final products. Great variability provides ample opportunity for selection and further improvement. It may not be feasible at this stage to select a particular individual with desired traits as no selections have been made in neem so far. A systematic study for tree improvement with a population of mother trees with desired traits should be undertaken by performing half-sib progeny trials and further selections by clonal propagations. The role of genetic makeup needs further research.

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