Effect of season and year on azadirachtin A and oil content in neem (*Azadirachta indica* A. Juss) seeds and relationship of azadirachtin A and oil content with rainfall, temperature and humidity

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In the present study, azadirachtin A and oil content on kernel basis have been estimated continuously for four years (2001–04) in 16 neem trees belonging to 13 districts of Uttar Pradesh, Madhya Pradesh and Rajasthan, and selected from progeny and provenance trials established in 1994 at the National Research Centre for Agro-forestry, Jhansi. These two biochemical parameters were also estimated in some tress which produce fruits in November. Coefficient of variation over the years was calculated for these parameters. Correlation studies of azadirachtin A and oil content were done with atmospheric temperature, rainfall and relative humidity. Results suggested that azadirachtin A was highly influenced by environmental factors compared to oil content in seeds. Positive and significant correlation was found for azadirachtin A and oil content with relative humidity, and negative and significant correlation with atmospheric temperature. Regions with comparatively low temperature (30–35°C) and high humidity (45–55%) during the period of flower initiation to fruit maturity favour high azadirachtin A and oil content in neem seeds. A one-year study on azadirachtin content in neem is not sufficient for arriving at any conclusions.

Keywords: Azadirachtin A, coefficient of variation, neem, oil content.

NEEM (*Azadirachta indica* A. Juss), a botanical cousin of mahogany, has been considered important for centuries due to its medicinal and insecticidal properties. Among various bioactive biochemicals present in the neem seeds, azadirachtin, a laminoid, is highly valued as an antifeedant replant and growth disturbing chemical against a variety of insect pests[1]. High azadirachtin content (> 0.50% by kernel weight) neem seeds are sold in the market at around Rs 10 per kg; otherwise the price is less than Rs 3 per kg. Due to low azadirachtin (< 0.50%) content in the seeds, only around 24% of the neem seeds are collected for processing to manufacture azadarachtin formulations[2]. Earlier studies suggested that the azadirachtin content is influenced by genotypes, regions, habitats and climatic conditions[3–7]. In these studies the set of genotypes tested was not similar in all geographical areas. Every region/habitat has a different set of genotypes. Therefore, it is difficult to conclude that the variation in azadirachtin content in seeds is due to environmental factors or genotypes, or the interaction of environment and genotypes. Therefore, in the present study 16 trees belonging to 13 districts of Uttar Pradesh, Madhya Pradesh and Rajasthan were selected from provenance and progeny trials of neem established in 1994, to study the effect of atmospheric temperature, relative humidity, rainfall and year on azadirachtin A and oil content in seed kernels over four years (2001–2004). In the same populations, besides the selection of 16 trees, seven trees were also identified in which flower initiation started in the first week of July and fruits matured in November (named as winter fruits). Fruits of these seven trees were collected in November 2005 (winter fruits) and July 2007 (summer fruits). Azadirachtin A and oil content of these fruits were also estimated.

For the estimation of oil content and azadirachtin A, greenish-yellow seeds (250 g) were picked directly from each selected tree. Fruits were depulped manually and washed thoroughly with clean water. Depulped seeds were dried in shade till constant weight. Next, 80 g kernels was taken from the seeds of each sample by manual decortication and converted into kernel powder using an electric grinder. Then 10 g powder was put in Whatman thimble and placed in Soxhlet unit of 250 ml R.B. flask containing petroleum ether (60–80°C) (200 ml) in condenser. The Soxhlet was kept overnight and the material was extracted. The extract was collected in a flask and organic solvent was completely removed by distillation under vacuum. The remaining oil content was weighed to get the oil yield. The solid residue left in the thimble was extracted with methanol (HPLC-grade) in the Soxhlet for 6 h at temperature below 60°C. The extract was collected in a flask and the volume of methanol extract containing azadirachtin A was measured and transferred to 250 ml corning bottle. A part of the sample (4 ml) was filtered in a culture tube through Durapore Hydralic filter 0.22 μm (Millipore) at the time of injecting into the HPLC (Shimadhu) apparatus. The sample (20 μl) was injected using autoinjector. Azadirachtin was separated on Phenmnex C-18 coloum (250 × 4.6 mn ID, 5 μl) using acetonitrite : water (40 : 60) @ 1 ml min^{-1} and the peak was monitored at 217 nm. Azadirachtin A content was determined following the methods of Kearney *et al.*[8] and Venkateshwarulu *et al.*[9]. Standard azadirachtin A (99%) was procured from Trifolio-MGmdH (Germany). A standard solution of azadirachtin A was prepared by dissolving 1 mg compound in 25 ml methanol (HPLC-grade).

Data on rainfall, atmospheric temperature and relative humidity during the period of flower initiation to fruit maturity were taken from Metrological Laboratory,

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Indian Grassland and Fodder Research Institute, Jhansi. Coefficient of variation for oil content and azadirachtin A over the years was calculated. Correlation coefficients for azadirachtin A and oil content with rainfall, minimum and maximum atmospheric temperature, relative humidity (II) during the period of flower initiation to fruit maturity were calculated.

Results showed reasonable range of variation between the trees for oil content and azadirachtin A (Tables 1 and 2). The range of variation was 0.089–0.800% for azadirachtin A and 34.56–50.00% for oil content on kernel basis. Coefficient of variation over the years was high for azadirachtin A compared to oil content for each tree. It ranged from 13.38% to 73.31%, with a mean of 36.93%. In the case of oil content it ranged from 1.98% to 12.34%, with a mean of 1.28% (Tables 1 and 2). These results indicate that azadirachtin A is highly influenced by environmental factors compared to oil content.

Table 1. Oil content (% on kernel basis) in seeds of selected heavy fruit-yielding trees along with coefficient of variation

		Oil content (%) in kernels					
Sample no.	Pedigree	2001	2002	2003	2004	Mean	Coefficient of variation (%)
18	F15L9T6	43.74	41.20	43.11	40.38	42.11	3.25
19	F15L10T14	42.25	39.56	35.82	42.98	40.15	6.99
20	F15L11T12	46.24	44.27	42.85	42.79	44.04	3.19
21	F15L11T20	37.94	41.69	34.56	42.77	39.24	8.26
25	F15L13T7	43.24	37.39	39.69	40.90	40.31	5.24
29	F15L16T8	43.91	42.35	41.90	43.67	42.96	1.98
31	F15L16T15	43.57	43.40	50.00	42.87	44.96	6.50
33	F15L18T16	40.17	37.45	35.97	48.91	40.63	12.34
34	F15L19T5	44.15	42.70	38.10	40.28	41.31	5.59
46	F15L26T22	43.08	43.02	47.56	46.92	45.15	4.67
11	F14L4T8	42.36	44.48	45.85	46.41	44.78	3.49
47	F14L7T8	43.27	44.34	46.39	43.99	44.50	2.60
13	F14L7T9	41.56	42.73	41.07	44.79	42.54	3.37
12	F14L7T7	43.09	43.81	48.24	41.25	44.10	5.82
14	F14L14T19	40.34	42.66	47.33	40.28	42.65	6.72
10	F14L16T19	47.93	38.80	38.27	43.09	42.02	9.26
Mean		42.93	41.87	42.29	43.27	42.59	1.28

Table 2. Azadirachtin A content (% on kernel basis) of selected heavy fruit-yielding trees along with coefficient of variation

		Azadirachtin A content (%) in kernels					
Sample no.	Pedigree	2001	2002	2003	2004	Mean	Coefficient of variation (%)
18	F15L9T6	0.330	0.096	0.132	0.189	0.187	47.71
19	F15L10T14	0.390	0.089	0.149	0.247	0.219	52.03
20	F15L11T12	0.340	0.115	0.127	0.182	0.191	46.94
21	F15L11T20	0.260	0.177	0.133	0.262	0.208	26.56
25	F15L13T7	0.320	0.182	0.190	0.271	0.241	23.88
29	F15L16T8	0.400	0.153	0.247	0.427	0.307	36.58
31	F15L16T15	0.270	0.117	0.179	0.219	0.196	28.52
33	F15L18T16	0.540	0.135	0.247	0.405	0.332	46.36
34	F15L19T5	0.420	0.119	0.151	0.153	0.211	57.68
46	F15L26T22	0.420	0.268	0.206	0.270	0.291	27.08
11	F14L4T8	0.310	0.182	0.111	0.229	0.208	34.78
47	F14L7T8	0.590	0.201	0.309	0.267	0.342	43.43
13	F14L7T9	0.350	0.216	0.178	0.376	0.280	30.21
12	F14L7T7	0.580	0.226	0.171	0.306	0.321	49.01
14	F14L14T19	0.250	0.197	0.261	0.289	0.249	13.38
10	F14L16T19	0.800	0.225	0.200	0.186	0.353	73.31
Mean		0.411	0.169	0.187	0.267	0.258	36.93

Table 3. Oil content and azadirachtin A (% on kernel basis) of seven neem trees that bear fruits during the winter season

	Azadirachtin A (% on kernel basis)			Oil content (% on kernel basis)		
Sample no.	Winter (2005)	Summer (2007)	Percentage of increase over summer	Winter (2005)	Summer (2007)	Percentage of increase/ decrease over summer
125	0.85	0.41	107.32	49.82	50.99	–2.29
126	0.68	0.29	134.48	48.93	47.6	+2.79
127	1.16	0.28	314.29	46.86	48.06	–2.50
128	0.83	0.17	388.24	43.55	45.14	–3.52
129	0.80	0.19	321.05	46.06	54.7	–15.80
130	0.72	0.37	94.59	44.91	48.26	–6.94
131	0.60	0.30	100.00	45.11	52.77	–6.41
Mean	0.81	0.29	179.31	46.46	49.65	–6.43
t (5%)	6.61*			2.07 (NS)		

*Significant at 1% level; NS, Not significant.

This is further confirmed by high average azadirachtin A content (0.81%) in the seeds obtained in November compared to those obtained in July (0.29%) in the same set of trees (Table 3). On the other hand, average oil content of seeds that mature in November was 46.46% as against 49.65% for seeds obtained in July, that is statistically at par with winter seeds. Therefore, preference should be given to breed varieties which produce fruits in November rather than July. Moreover, seeds obtained in November are less infected by fungus compared to those obtained in July. Hence reduce the chances of aflatoxin in seeds. High value of coefficient of variation for azadirachtin suggests that it is not desirable to draw any conclusion on any aspect related to azadirachtin content based on a single-year data. To identify high azadirachtin content genotype/genotypes of neem, it is essential to test the genetic materials in at least 2–3 locations over 3–4 years. Contrary to this, identification of high oil content genotype/genotypes is comparatively easy.

Correlation coefficients were estimated for azadirachtin A and oil content with rainfall, minimum and maximum atmospheric temperature, and relative humidity (II) during the period of flower initiation to fruit maturity. Correlation studies have shown that there was negative and significant correlation of azadirachtin A with maximum (–0.9769) and minimum (–0.8891) atmospheric temperature. This suggests that high temperature (more than 40°C) during the period of flower initiation to fruit maturity reduces azadirachtin A in seeds. On the other hand, positive and significant correlation of azadirachtin A was observed with relative humidity (II) (0.9930). Thus, high humidity favours high azadirachtin content. Rainfall had a positive but not significant correlation (0.7106) with azadirachtin A, though the value of correlation coefficient was high.

Results of correlation studies between oil content and metrological parameters are similar to those of azadirachtin A content in seeds. Oil content had negative and significant correlation with maximum temperature (–0.9639), and positive and significant correlation with relative humidity (II) (0.9930). Positive and non-significant correlation (0.5721) was found with rainfall. Thus, regions with comparatively low temperature (range 30–35°C) and high humidity (45–55%) during flowering to fruit maturity are more suitable to obtain neem seeds with high azadirachtin A and high oil content.

Several researchers have reported variation in azadirachtin content in neem seeds due to various factors. Rengasamy and Parmar[5] reported that ecotypes belonging to coastal, arid and semi-arid ecosystems showed high azadirachtin A than those from the sub-humid regions. Similarly, marked variation in azadirachtin content in neem seeds of various countries has been observed by Ermel *et al.*[3]. Seeds from Nicaragua and Indonesia have more azadirachtin followed by those from India, Burma and Mauritius. Venkateswarulu *et al.*[9] observed marked variation between different genotypes of the same location, as well as different locations, but there was no relationship between azadirachtin A content and rainfall, humidity and temperature. Seasonal variation in azadirachtin A, B and F was reported by Sidhu and Behl[6]. Winter stress appears to favour synthesis of azadirachtin B and F in seeds. Kaushik *et al.*[7] reported that azadirachtin was affected by climate and habitat. Annual variation in azadirachtin content was significant. The highest azadirachtin content was recorded in neem tree populations growing in the southern parts of India.

In all the above studies, seeds did not belong to the same genotypes tested in various locations and the studies were not conducted over several years. Thus, it is difficult to conclude whether variation in azadirachtin content in seeds is due to climatic factors, soil types, genetic make-up or a combination of these. On the other hand, in the present study genotypes were the same, planted in the same location, and the variation observed might be due to climatic factors. It can be concluded that seeds of neem

obtained in November have 2–4 times more azadirachtin A than those obtained in July. Thus, emphasis should be given to identify genotypes of neem that will give fruits in November, rather than in rainy season. These seeds will also be less infected with fungi that deteriorate the quality of seeds.

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Modelling of a magnetic anomaly in east Ganga basin and its implications on the tectonics of the region

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Magnetic data around Muzaffarpur in the eastern part of the Ganga basin display a conspicuous magnetic anomaly in an otherwise relatively smooth magnetic terrain. The anomaly with intense amplitude of about 1500 nT is located at the junction of the west Patna Fault and the Sitamarhi Fault. The low–high axis of this anomaly is oriented at 45°E, suggesting that the source possesses remanent magnetism. Magnetic modelling reveals that the source, located at a depth of 4200 m, is polarized at an inclination of 10°S and declination of 45°E. This implies that the causative source is emplaced when this part of the continent is located 5° south of the equator and is tilted 50°E with respect to the present position, describing a moment of the northward journey of the Indian plate after its break-up from Antarctica.

Keywords: Aeromagnetics, magnetic anomaly, remanent magnetism, tectomics.

THE Ganga basin occupying a vast area of about 300,000 km^2 in the northern part of India is bounded by the Himalayas in the north and the Aravallis in the west. The Vindhyans and the Bundelkhand granite delimit its boundary to the south, whereas the Chota Nagpur Plateau serves as its eastern boundary. As the entire area is covered under a thick blanket of alluvium, the subsurface geology[1] (Figure 1) was inferred from geophysical data and drilling. The area was covered by airborne magnetic surveys[2–4], and ground magnetic, gravity and seismic surveys[5–8]. The geophysical data over the Ganga basin was interpreted in detail in terms of subsurface structures[2,3,6,7].

Although the magnetic anomalies over a thick sedimentary sequence of the Ganga basin are expected to display gentle gradients, data in the eastern part of the basin north of Muzaffarpur exhibit intense anomalies. The vertical magnetic intensity map[4,5] (Figure 2) of this area shows intense anomalies (marked A and B) of about 1500 nT, spreading over an area of about 30 × 40 sq. km. Although both the anomalies appear to be similar, data coverage of anomaly B is sparse and hence only anomaly A is used in modelling and interpretation as described in the following.

For magnetic modelling, it is essential to know the nature of magnetic anomalies which depend not only on

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