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## Azadirachtin Variation of Six Provenances of *Azadirachta excelsa* (Jack) Jacob.

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**Abstract:** In this study, azadirachtin concentrations of *A. excelsa* from six provenances viz, Bukit Lagong (Selangor), Pengkalan Arang (Terengganu), Narathiwat (Thailand), Manong (Perak), Semengoh (Sarawak) and Pasir Mas (Kelantan) were investigated. The samples were extracted using Soxhlet apparatus and the crude extracts were analysed using High Performance Liquid Chromatography (HPLC). The mean value of azadirachtin concentrations obtained ranged from  $2.33 \pm 0.76$  ppm  $g^{-1}$  (Pengkalan Arang, Terengganu) to  $6.26 \pm 0.87$  ppm  $g^{-1}$  (Bukit Lagong, Selangor). The highest value of azadirachtin ( $21.94$  ppm  $g^{-1}$ ) was obtained from an individual tree from the Manong, Perak provenance whereas the lowest value ( $0.37$  ppm  $g^{-1}$ ) was recorded from Pasir Mas, Kelantan provenance. Azadirachtin concentration from Bukit Lagong and Manong provenances was significantly higher at 5% level than provenances from Pengkalan Arang, Narathiwat, Semengoh and Pasir Mas. The highest yields of azadirachtin were not restricted to a specific provenance but they came from single trees of different origin. Thus, breeding for high-yielding azadirachtin tree would be best based on selection of individual tree within the provenance and inter provenances.

**Key words:** *Azadirachta excelsa*, azadirachtin, provenances, sentang

### INTRODUCTION

*Azadirachta* is a member of Meliaceae, which comprises of 50-52 genera with about 550 species and is distributed throughout the tropics and subtropics. It is locally known as Sentang or kayu bawang in Peninsular Malaysia, Runggu in Sarawak, Limpaga in Sabah, Sadao-thiam in Thailand and Bie in Papua New Guinea (Schumutterer and Doll, 1993; Norani, 1997). It is a fast growing species with an average girth growth rate of 8-10 cm per year in Thailand. It yields a very attractive reddish, high quality timber which can be used for light construction, furniture, interior finishing, paneling, matches, boats, decorative engraving, MDF and pulp production (Norani, 1997). In addition to its timber utilisation, various parts of the tree have been used as traditional medicine (Chungpongse and Buranatham, 1991). Extract from the leaves of *A. excelsa*, which contains mainly azadirachtin has shown highly promising effect in insecticidal and growth regulating, as antimalaria, antiseptic and antimicrobial agent, pharmaceutical and medicinal uses.

Azadirachtin is a tetranortriterpenoid plant limonoid which has potent antifeedant, growth and reproductive regulating properties. Various studies conducted have shown that azadirachtin yields good results in combating

a wide range of insects which includes phytophagous insects from the order Lepidoptera, Diptera, Orthoptera, Hemiptera, Coleoptera and Hymenoptera (Arnason *et al.*, 1985; Mordue *et al.*, 1985). Apart from these, it is also found effective when it deals with certain nematodes, fungi, viruses and protozoa. Azadirachtin also has the ability to act as a powerful spermicide according to Sadre *et al.* (1983). Information from the Neem Association, has also shown that extract from neem or *Azadirachta indica*, one of the closely related species to *A. excelsa*, had been effective against heart diseases, high blood pressure, psoriasis, eczema, diabetes, cancer, periodontal diseases and nerve disorders. Interestingly, neem extract also contains mainly azadirachtin.

### MATERIALS AND METHODS

**Sampling:** Collection of leave samples were done in June 2000 from a three-year-old provenance trial of *A. excelsa* at Rantau Panjang Forest Reserve, Batu Arang, Selangor. The trial is located about 70 km Northwest from University Putra Malaysia (UPM). Plant samples were taken from six provenances namely: Bukit Lagong (Selangor), Pengkalan Arang (Terengganu), Narathiwat (Thailand), Manong (Perak), Semengoh (Sarawak) and Pasir Mas (Kelantan). Leaves were sampled randomly from each provenance.

Eight to twelve leaflets were taken randomly from different branches of each tree and were placed inside a labeled paper envelope. The samples were stored at 4°C before use.

**Extraction:** The leaf samples were placed inside a paper envelope and were oven-dried at 40°C for three weeks. The dried samples were then grounded into a fine powder, to aid extraction. The samples of each powder were weighed and placed inside a cellulose extraction thimble. The samples were refluxed using a set of Soxhlet apparatus for 2 h using 150 mL acetone. The suspended extract was dried using a rotary evaporator at 60°C. Finally the resultant residue was resuspended using 4×1 mL aliquots of HPLC grade methanol. The sample solutions were stored in refrigerator at 4°C.

**HPLC analysis:** Analytical High Performance Liquid Chromatography (HPLC) was performed using a UV absorbance detector and C 18 column set at 218 nm, flow rate 1 mL min<sup>-1</sup> and pressure 160-180 kg in<sup>-2</sup>. The solvent used was Acetonitrile: water (47:52). Four concentrations of azadirachtin standards were used viz., 25, 50, 75 and 100 ppm. These were used to calibrate a standard curve. Twenty microliter of each sample was injected into the HPLC machine. The concentration of each sample was calculated based on the standard curve.

**Data analysis:** The variations of azadirachtin of the six provenances were analysed using analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

There was significant difference between the provenances with regards to azadirachtin content. Azadirachtin concentration of *Azadirachta excelsa* of samples from Bukit Lagong (Selangor) and Manong (Perak) from provenances trial established at Rantau Panjang Forest Reserve were significantly higher than the samples from Pengkalan Arang (Terengganu), Narathiwat (Thailand), Semengoh (Sarawak) and Pasir Mas (Kelantan) provenances. The mean values of Azadirachtin

Table 1: Mean values of Azadirachtin concentrations from various provenances

Provenances	Mean Azadirachtin concentration (ppm g <sup>-1</sup> dried leaf)
Bukit Lagong, Selangor	6.26±0.87 <sup>a</sup>
Manong, Perak	5.96±1.30 <sup>b</sup>
Pasir Mas, Kelantan	3.04±1.26 <sup>b</sup>
Narathiwat, Thailand	2.92±0.94 <sup>b</sup>
Semengoh, Sarawak	2.37±0.54 <sup>b</sup>
Pengkalan Arang, Terengganu	2.33±0.76 <sup>b</sup>

Means with the same letter are not significantly different at p<0.05.

concentration ranged from 2.33±0.76 ppm g<sup>-1</sup> (Pengkalan Arang) to 6.26±0.87 ppm g<sup>-1</sup> (Bukit Lagong) (Table 1).

The highest value of Azadirachtin (21.94 ppm g<sup>-1</sup>) of individual trees was obtained from Manong provenance, whereas the lowest value (0.37 ppm g<sup>-1</sup>) was recorded from Pasir Mas provenance. Azadirachtin concentration of *Azadirachta excelsa* trees from these provenances, grouping the sample concentrations from the six provenances into six classes, indicated that most of the samples produced less than 12 ppm g<sup>-1</sup> Azadirachtin per gram of dry leaf sample (Table 2).

Not all dried leaves samples from these six provenances contained Azadirachtin. Dried leave samples from Bukit Lagong and Manong provenances gave the highest percentages containing Azadirachtin, whereas dried leave samples from Pengkalan Arang gave the lowest percentage (Table 2).

In this study, Azadirachtin concentration of *Azadirachta excelsa* (Sentang) from Bukit Lagong and Manong provenances were significantly higher than Pengkalan Arang, Narathiwat, Semengoh and Pasir Mas provenances, at 5% level. Work done by Nor Aini and Chin (1997) showed that Azadirachtin concentration of sample from Pahang, Kedah, FRIM and Perak were significantly higher than UPM sample at 5%. There were no significant differences among the provenances from Bukit Lagong and Manong and there was also no significant difference between the provenances from Pengkalan Arang, Narathiwat, Semengoh and Pasir Mas. This is because leaves samples were taken from an experimental plot and not from the natural stand population which usually possessed narrow genetic

Table 2: Variation of Azadirachtin in different *A. excelsa* dried leave samples from six different provenances

Provenances	Sample that contains Azadirachtin out of 15	Highest value of Azadirachtin recorded (ppm g <sup>-1</sup> )	Lowest value of Azadirachtin recorded (ppm g <sup>-1</sup> )	% of samples that contains Azadirachtin
Bukit Lagong, FRIM	14	11.59	2.22	93.33
Pengkalan Arang, Terengganu	8	8.17	1.21	53.33
Narathiwat, Thailand	11	11.43	1.17	73.33
Manong, Perak	14	21.94	2.42	93.33
Semengoh, Sarawak	13	5.49	0.47	86.66
Pasir Mas, Kelantan	10	14.21	0.37	66.66

variation. Provenance of Bukit Lagong and Manong were believed to originate from the same source and this resulted in no significant difference in azadirachtin production from these two provenances.

Generally, genetic information in the natural population reaches a maximum within the natural range where the species is in its optimum (Kleinschmit, 1974). In general, genetic variation is greater in natural population compared to cultivated population (plantation). Van Wyk *et al.* (1995) reported that aloin content was clearly related to provenances and the mean variation of aloin content in *A. ferox* obtained from different provenances were very high. However, the mean values of Azadirachtin obtained from six provenances of *A. excelsa*, of this study were small in variation. This is because the samples of *A. ferox* were taken from natural populations whereas samples of *A. excelsa* were taken from an experimental plot for this study. Singh (1986) also found a big difference in antifeedant activity between different provenances of neem. Neem growing in arid areas possessed much higher antifeedant activity than those growing in coastal areas, suggesting that environmental conditions do play a role in quantifying the Azadirachtin produced, perhaps in terms of differences in the physical environment such as temperature and humidity or may be due to differences in the biological environments such as predator levels.

Azadirachtin concentration in *A. excelsa* is a quantitative value that will be influenced by environment and not merely by the genetic substances in the plant itself. The age of this provenance trial is three years old and it is considered that the period of time is long enough for the trees of various provenances to adapt to the given environment.

The crude extracts of these trees contained only a small portion of azadirachtin compared to other components. This is because some amount of energy is needed in producing such defensive chemicals. Based on the principle of cost benefits and tradeoff in plants (Rhoades, 1979), they will only produce such chemicals when in need. In other words if more secondary product or in this context, the defensive chemical is produced, then the trees will reduce their growth rate and fecundity. Therefore plants will try to produce just enough Azadirachtin for protective purposes and this accounts for the very low concentration of Azadirachtin detected in the samples.

As indicated above, the highest value of Azadirachtin (21.94 ppm g<sup>-1</sup>) was obtained from an individual tree from Manong provenance whereas the lowest (0.37 ppm g<sup>-1</sup>) was recorded from Pasir Mas provenance. The unpredictable variability of Azadirachtin

contents present in the trees from different provenances could confuse the insects (pests). This is because predation of the insects on a tree with higher Azadirachtin concentration in the population will deter the insects from predated other trees of the same species. This could benefit the whole population in the form of growth performance. In this study, it was found that the majority of the trees opt/chose to produce smaller amounts of azadirachtin perhaps cheating other trees to produce more by gaining additional protection without using extra energy.

In this study, the mean concentrations and individual concentration of Azadirachtin detected were lower than the ones reported by Nor Aini and Chin (1997), Dobbins and Deag (Unpubl). The environmental factors such as the geographical information of site, method of sampling preparation, Azadirachtin characteristics and the physiology of the trees themselves were among some of the factors affecting the variation.

It was also observed that there was deficiency in nutrients in this provenance trial based from the phenotypic expression of the trees. The production of Azadirachtin would affect the growth rate of the tree, for instance, if the production of azadirachtin is high, then the growth rate will be reduced. In an environment where the resources are limited, the trees must strike a balance between production of protective compounds such as Azadirachtin and also their fitness. In such environment, survival is more important than protection, so plant will tend to improve their vegetative growth (shoot and root growth) rather than producing defensive chemicals so that they have more advantages to compete with other plants in the same site.

Azadirachtin is sensitive to heat and upon storage time. It was observed that Azadirachtin gets degraded over time. In fact, the Azadirachtin standards (with concentrations of 25, 50, 75 and 100 ppm) used in this study got totally degraded after one month despite being kept in a refrigerator at 4°C. This explains why azadirachtin detected in the present samples were lower than the one obtained from previous studies and many of present samples actually did not have Azadirachtin.

Leave samples from the Bukit Lagong (Selangor) and Pengkalan Arang (Terengganu) provenances generally produced the highest and lowest mean values of azadirachtin. Although the amount in term of concentration of azadirachtin produced is affected by the provenance or origin of the sources but the yield extracts of azadirachtin are not restricted only to a specific provenance but depending on specific individual trees of the different origin.

Not all dried leaves samples examined contain the biologically active azadirachtin and the concentration of azadirachtin obtained from the samples were very low. Azadirachtin concentration in dried leaf extract was found to degrade over time. Long term investigation are needed to verify as to whether environmental factors such as climatic condition, soil temperature, rainfall as well as harvesting time and storage conditions (Schneider, 1986; Shirish *et al.*, 1995; Ermel *et al.*, 1986) could affect the amount of azadirachtin produced.

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