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Regional And Habitat Variability In Azadirachtin Content Of Indian Neem (Azadirachta Indica A. Jusieu)

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I. Introduction

Neem (*Azadirachta indica* A. Jusieu) is an evergreen, multi-purpose tree found in the Indian subcontinent and Southeast Asian countries. Every part of the tree is useful in one way or the other (1). The seed is an important source of vegetable oil and biopesticidal compounds. Neem seed kernel consists of (2, 3) azadirachtin and other limonoids such as nimbin, salanin(4)and meliantriol (5,6). However, azadirachtin is the most important limonoid and is known to possess anti-feedant, attractant, repellant (7), growth disrupting (8) and larvicidal properties against numerous pests (9).

It is essential to understand the regional and habitat variations in azadirachtin content of neem trees for identification of region-specific elite trees and also to understand the factors affecting the synthesis of azadirachtin. Limited studies carried out on azadirachtin variation suggest that azadirachtin content is influenced by climatic conditions. Ermel *et.al* (10) assessed, for the first time, the wide variability of azadirachtin content in neem seeds of different countries and found that the highest yield of azadirachtin content per seed kernel is not restricted to a specific country but is distributed in single trees of different origin. Few reports are available on azadirachtin content variation in different ecotypes and provinces of India (11).

This article presents the findings of the study of azadirachtin variability observed in different agroclimatic regions and habitats of Tamilnadu.

II. Experimental details

Collection of neem seeds

Neem seeds were collected from 15 candidate plus trees selected from 3 districts of Tamilnadu (Figure 1). Fully ripe, yellow fruits were collected directly from branches of individual trees. Fruits were depulped manually and washed thoroughly with clean water to remove traces of pulp from the seed coat. The depulped and washed seeds were dried in the shade

Chemical analysis

100 g of neem powder was placed on the thimble and placed in the soxhlet apparatus. 500 ml of selected solvents were placed in a round bottom flask and assembled for soxhlet extractor then, the distillation process has begun. After completing the extraction process, the solvent and extractor were placed on water bath to evaporate the solvent.

Azadirachtin content was estimated using calibration curves. A standard solution of azadirachtin (1000 µg/ml) was prepared by dissolving 10 mg of the compound in 10 ml of HPLC-grade acetonitrile. Serial dilutions were made in the range of 100–10 µg/ml to plot the calibration curve. The standard solutions were stored at –20°C. The value of azadirachtin content has calculated based on calibration and expressed as ppm (µg/g of the kernel weight).

Statistical analysis

The azadirachtin sample has clustered on the basis of geographical region of collection for analyzing the results statistically. Data has analyzed by employing one-way ANOVA and Duncan Multiple Range Test (DMRT) at 5% significance level.

III. Results And Discussion

Azadirachtin content in the seeds collected from the three different districts of Tamilnadu i.e. Chengalpattu, Vellore and Villupuram (Figure 1) revealed large, overall variations ranging from 200 to 3000 ppm (µg/g) of the seed kernel. Such a wide variability is expected due to environmental effect. The average azadirachtin content of these accessions has 2056 ppm (µg/g of the seed kernel) (Figure 2). About 7 of the 15

samples recorded more than 2,000 ppm (µg/g) of azadirachtin content (µg/g of the seed kernel). A majority of these samples were from the Vellore district.

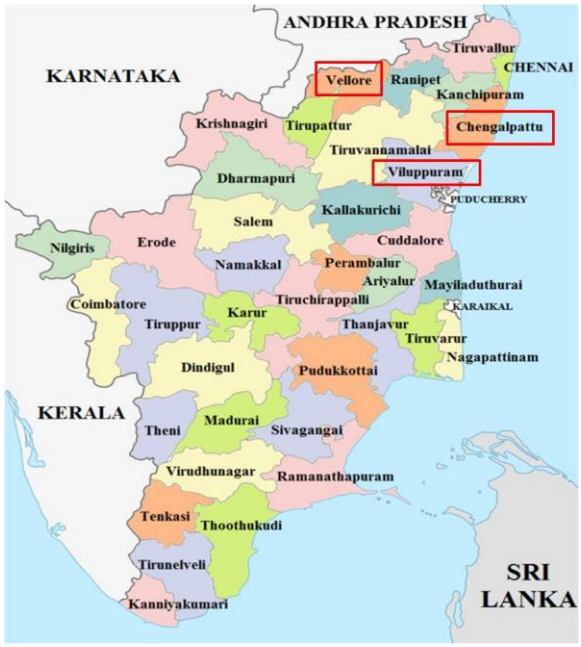


Figure 1: Map of Tamilnadu representing the extent of collection from different parts as boxed area

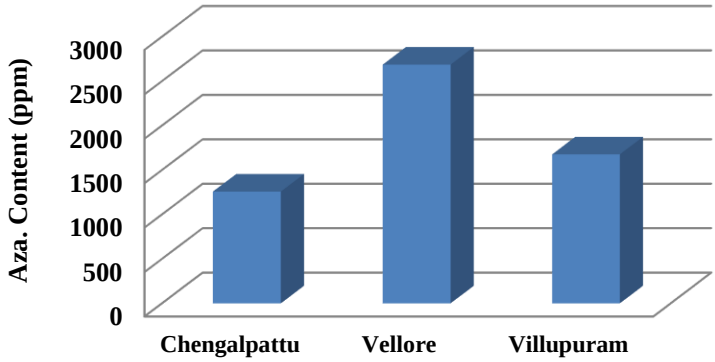
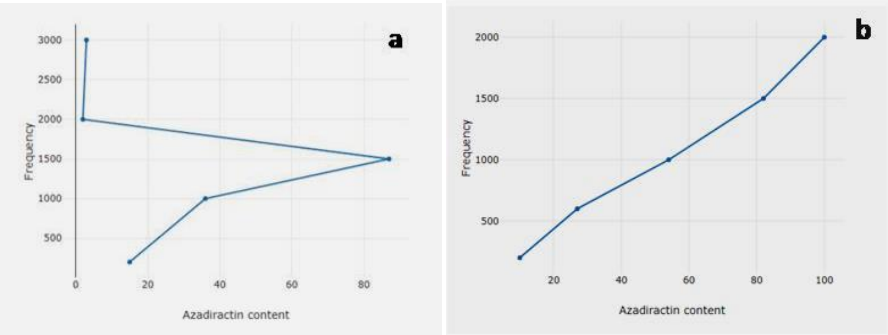


Figure 2: Average azadirachtin content (µg/g of seed kernel) in neem seeds

Based on azadirachtin content within the samples obtained from each district, frequency with cumulative percentage has prepared (Figure 3a – 3c). The percentage further reveals the effect of geographical location on the distribution of azadirachtin content. In Vellore, nearly 60% of the sample were in the range <1000 - 3000 ppm azadirachtin content, whereas in Villupuram, nearly 25% of the sample were in the range <800 - 2000 ppm and in Chengalpattu, nearly 15% of the sample were in the range <200 - 1500 ppm (Figure 4).



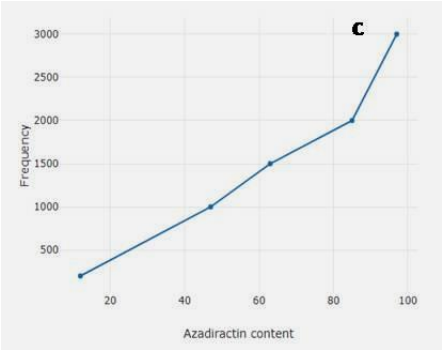


Figure 3: Frequency for azadirachtin content (µg/g of the kernel) for (a) Chengalpattu, (b) Vellore, (c) Villupuram

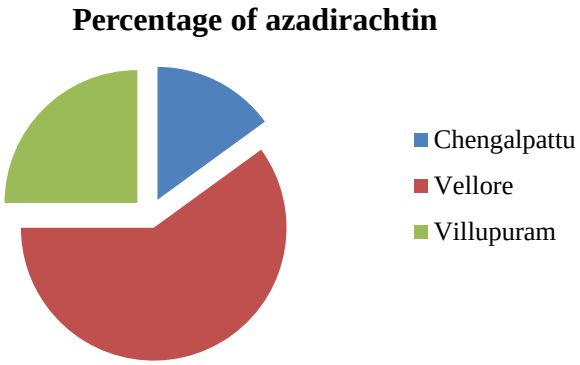


Figure 2: Average azadirachtin content (µg/g of seed kernel) in neem seeds

IV. Effect Of Growth Period

The growth period available for a plant species affects the total photosynthetic output and thus production of secondary metabolites. In neem, flower initiation starts in February–March, flowers bloom in April and fruits ripen in July–August. In order to study the effect of the growth period on azadirachtin content, the samples has divided into three classes on the basis of the growth period with <90, 90–150 and 150–180 days of growth period, and data for these classes are analysed statistically. It is evident from statistical analysis (Table 3) that azadirachtin is significantly high in trees growing during the 90–150 days of growth period. This was closely followed by a 150-180 days of growth period, while shorter growth period (<90) was found to be least favourable for optimum production of azadirachtin. Thus, a 90–150 days growth period has found optimum for synthesis of azadirachtin. This indicates that shorter growth periods are not favourable for yielding high azadirachtin content.

The present findings are based on extensive analysis carried out by different districts of Tamilnadu. A small proportion of samples are recorded distinctly high azadirachtin content. Trees yielding high azadirachtin content are extremely valuable for plantation programmes. The study further reveals that geographical conditions are most suitable for greater synthesis of azadirachtin in neem trees.

India has produced quality neem seeds of approximately 660,000 tonnes (12). Looking at the growing demand for biopesticides in integrated pest management, and organic modes of agriculture, more neem trees should be grown as block plantations or as agroforestry component in farmers' fields to increase the availability of seeds. On average, one tree yields 30–50 kg seeds/yr. Its productivity can be further increased by selecting superior genotypes. Therefore, from the present study, high azadirachtin-bearing trees need to be conserved and multiplied for better utilization. Performance of the selected trees over a longer period of time, and under different agroclimatic conditions through multi-location trials will be evaluated in future.

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