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# Variation in Oil Composition of *Thevetia Peruviana* Juss 'Yellow Oleander' Fruit Seeds

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**ABSTRACT:** *Thevetia peruviana* J 'Yellow Oleander' is a potential oil seed (63% oil) and good alternative protein source (37%) for livestock feeds. The plant remains an ornamental plant because of the high level of toxins in the seeds. *Thevetia peruviana*, a tropical oil-seed plant can be grouped into four varieties based on the number of kernels per fruits; two varieties based on the colour of the flower and three varieties based on the geographical locations. The two-seed variety is the richest in oil (63%); examinations of the oils for variations based on the geographical location show that plants growing in the middle region of Nigeria have the best oil properties. The fatty acid composition of purple colored flowers is slightly higher in unsaturated fatty acids; and oils from seeds of the driest zone was higher in level of total unsaturation of the oil (62.7%) @JASEM

*Thevetia peruviana* J belongs to the order apocynales and Apocynaceae family. It is a native of tropical America; especially Mexico and West Indies, but has naturalized in tropical regions worldwide. It is cultivated and remains an ornamental shrub in spite of the high oil content (63%) and favourable protein content (37%) of the seed. The defatted seed cake however has a high level of toxicity (Ching -Chang *et al.*, 1966; Bisset, 1963; Chen and Henderson, 1963; Aleshkina and Berezhinskaya, 1963; Sticker, 1970). It is likely that the attention given to toxins has distracted interest from proper research of the oil and protein that would have promoted its industrial and domestic potentials. Several feeding experiments (Atteh *et al.*, 1990; Atteh *et al.*, 1995) and thermal studies (Ibiyemi *et al.*, 1995) have shown that the oil has a very good replacement value for orthodox domestic vegetable oils. Pot experiments (Ibiyemi and Faloye, 1990) show that the plant responds well to nitrogenous fertilizer and its response to calcium and phosphorus follows the normal pattern for most plants (Ibiyemi and Popoola, 1991; Manchanda *et al.*, 1982; Prasad and Shina, 1981). Oil from *T. peruviana* will compete effectively with orthodox oils if its plantations would be developed. This report does not provide good evidence for the chemotaxonomy variability of the plant. They, however, serve as take-off board that shall freshly stimulate necessary collaborative research by chemists with horticulturists or geneticists for future development of the plant plantations for specific utility values of *T. peruviana* plant.

## MATERIALS AND METHODS

This study carves Nigeria into three zones along the major vegetation belts: thick forest, dispersed forest and grassland savannah. These three geographical zones, each representing a rainbelt, were selected for collection of fruits for the study. Three plants were randomly selected for each zone: Ilorin and Edidi represent a region of medium rainfall, spreading over 5 months per year; Enugu has a rainfall spreading over 7 months, while Zaria represents the driest region with about 4 months of rainfall per year. Two plants with purple-color- flower available from Enugu were studied for flower-type. About 300 fruits that had ripened were collected for each plant.

Variety in this report implies: Fruits with the same number of kernels per fruit, Fruits from the same geographical location, Fruit from plants with same color of flower. Kernels from fruits of each variety were crushed to obtain a paste, which was exhaustively extracted by Soxhlet method with petroleum ether (60 to 80°C) to obtain the oil. The proximate analytical properties: saponification value, iodine value, peroxide value, acid value, unsaponification value of each varietal - type oil were determined by Standard Official and Tentative Methods of Oil Chemists' Society (AOCS 1979). The fatty acid composition of each was determined by Gas Liquid Chromatography (GLC) analysis of the methyl ester. Two drops of each oils were treated with 0.1 ml of 8% $BF_3$/methanol solution in a screw capped centrifuge tube. This was then flushed with nitrogen and heated at 100°C for one hour. Analysis was carried out using Perkin-Elmer 82440 capillary gas chromagography (GC) fitted with a Flame Ionisation Detector (FID) and a flexible fused silica open-tubular column 30 x 0.32mm internal diameter, coated with OMEGAWAX 320. The program of column temperature was initially 185°C for 18 minutes; them increased at 3°C/min to 230°C. Helium was used as carrier gas at a pressure of 12psi. The injection pot and detector were maintained at

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250°C and 270°C respectively. Peaks were identified using authentic standards. The percentage of each fatty acid component was calculated as the peak area percent of the total of all fatty acids. Statistical analysis of the results of the fatty acid methyl esters is by the Duncan grouping procedure while other data were analyzed by analysis of variance (ANOVA) method.

## RESULTS AND DISCUSSION

*T. peruviana_* plants have been grown as an ornamental plant in homes, schools and churches for over fifty years in Nigeria, by missionaries and explorers. It grows more abundantly wildly and as ornamental flower hedge in the wetter southern region. Similarities in the results of analysis of soil samples for nitrogen, phosphorus and potassium for the four locations suggest that soil effect does not contribute to any variation in the results of our studies. Varietal differences therefore shall be attributed to either climatic factors or fruit - type.

*Thevetia peruviana* plants grown as hedge produce more than 400-800 fruits per annum depending on the rainfall and plant age. The long break in rainfall experienced in July and August in Ilorin and Edidi in 1996 caused a significant statistical decrease in the number of fruits each plant produced that year (Table 1a). Plants grown in the middle-belt rain region produced flowers about a year earlier than those from Zaria, the drier region. Fruit population distribution (Table 1b) for variety based on geographical location follows the same pattern. There is a significant variation in the population distribution for each of the four-seed variety. The fruit – type however follows the same distribution pattern. Location by type i.e. the relative behaviour of each fruit – type distribution is uniform regardless of the location i.e. the best fruit – type in one location will be the best in another location and vice versa. The population of the one-seed variety in Zaria is significantly lower than the population of the same variety in other regions except Enugu. The population of the 2-seed variety in Zaria is significantly different from the population in Ilorin. The population of the 4-seed variety is significantly low in Zaria.

This result could suggest that the fruit varieties based on number of kernels is hereditary and preserved under different climatic conditions. For purpose of plantation development, both one-seed and two-seed varieties are recommended to plant breeders for possible trials. The one-seed and two-seed plants produce more fruits per hectare, and although the two-seed plant produces fewer fruits than the one-seed, it however produces more oil than the one-seed plant and this will be to advantage.

The statistical analysis of each of the saponification value, iodine value, peroxide value, etc (Table 2b) of the oils show that there are varying significant differences among the fruit– types. There are less significant variations for the refractive index and specific gravity for the oil samples. Each variation does not follow a definite pattern.

**Table (1a)** Seed average population-distribution, 1994-1998 in one geographical location

| Location | Number of Fruits per plant | | | | |
|---|---|---|---|---|---|
| | 1994 | 1995 | 1996 | 1997 | 1998 |
| Ilorin | 765 ± 13.80 | 760 ± 11.00 | 651 ± 12.01 | 771 ± 11.14 | 777 ± 19.8 |
| Edidi | 774 ± 11.00 | 770 ± 8.33 | 666 ± 26.69 | 761 ± 11.14 | 764 ± 6.24 |

Each value is a mean of data from at least three determinations.

**Table (1b)** Population- distribution of Fruit Variety by region in Nigeria

| Geographical Location | Fruit Type (Number of Kernel per fruit) | | | |
|---|---|---|---|---|
| | One-Seed | Two- Seed | Three-Seed | Four-Seed |
| Ilorin | 127 ± 3.51 | 74 ± 2.65 | 85 ± 5.29 | 26 ± 3.6 |
| Edidi | 128 ± 2.00 | 71 ± 2.00 | 81 ± 3.06 | 31 ± 1.53 |
| Zaria | 120 ± 7.00 | 67 ± 3.60 | 79 ± 4.04 | 22 ± 4.16 |
| Enugu | 126 ± 2.65 | 71 ± 2.08 | 81 ± 1.53 | 31 ± 1.53 |
| Enugu (Purple) | 129 ± 0.58 | 69 ± 1.00 | 83 ± 2.00 | 30 ± 1.53 |

Each value is a mean of data from at least three determinations.

**Table (2a).** Performance of plant varieties by Fruit –Types

| Fruit Analysis | Fruit-Type (Number of Kernel per Fruit) | | | |
|---|---|---|---|---|
| | One-Seed | Two-Seed | Three-Seed | Four-Seed |
| Kernel/Fruit (%) | 31.3 ± 4.4 | 38.8 ± 7.1 | 33.6 ± 4.4 | 21.8 ± 2.2 |
| Oil Yield (%) | 61.1 ± 1.14 | 63.8 ± 0.61 | 58.7 ± 0.7 | 59.0 ± 1.0 |

Each value is a mean of data from at least three determinations.

**Table (2b)** Summary of Result for analysis of Oils from Fruit Type Varietal

| Analytical Parameter | Fruit-Type (Number of Kernel per Fruit) | | | |
|---|---|---|---|---|
| | One-Seed | Two-Seed | Three-Seed | Four-Seed |
| Saponification Value (%) | 121.05 ± 1.38 | 124.29± 3.79 | 120.53 ± 0.84 | 120.26 ± 1.13 |
| Unsaponifiable Value (%) | 0.27 ± 0.026 | 0.21 ± 0.026 | 0.42 ± 0.036 | 0.38 ± 0.026 |
| Free Fatty Acid | 0.49 ± 0.035 | 0.44 ± 0.056 | 0.57 ± 0.056 | 0.51 ± 0.015 |
| IodineValue | 78.6 ± 0.46 | 78.0 ± 0.87 | 79.8 ± 0.82 | 82.0 ± 0.11 |
| Peroxide Value | 3.2 ± 0.2 | 3.6 ± 0.17 | 3.8 ± 0.17 | 3.8 ± 0.2 |
| Refractive Index | 1.461 ± 0.001 | 1.463 ± 0.002 | 1.462 ± 0.002 | 1.462 ± 0.002 |
| Specific Gravity | 0.926 ± 0.003 | 0.923 ± 0.001 | 0.927 ± 0.004 | 0.933 ± 0.005 |

Each value is a mean of data from at least three determinations.

**Table (3)** Summary of Results from analysis of fruits from four different Nigerian geographical locations

| ANALYTICAL PARAMETER OF FRUIT | SAMPLE SOURCES | | | |
|---|---|---|---|---|
| | ILORIN | EDIDI | ENUGU | ZARIA |
| Number of Fruit per Plant | 600-750 | 600-750 | 650-800 | 400-650 |
| Weight of Fruit[a] | 5.5 ± 0.26 | 5.8 ± 0.17 | 6.1 ± 0.2 | 4.9 ± 0.15 |
| Kernel per Fruit[b] (%) | 36.7 ± 6.14 | 31.5 ± 6.6 | 39.3 ± 5.1 | 33.8 ± 7.3 |
| Oil Yield (%)[b] | 63.5 ± 1.32 | 63.3 ± 0.61 | 61.8 ± 0.82 | 64.7 ± 0.52 |
| Saponification Value (%)[b] | 123.1 ± 0.36 | 123.5 ± 0.44 | 124.7 ± 0.56 | 127.3 ± 0.72 |
| Unsaponification Value (%)[b] | 0.35 ± 0.017 | 0.38 ± 0.026 | 0.31 ± 0.01 | 0.30 ± 0.02 |
| Free Fatty Acid (%)[b] | 0.63 ± 0.017 | 0.62 ± 0.017 | 0.61 ± 0.02 | 0.63 ± 0.017 |
| Iodine Value[b] | 79.3 ± 0.26 | 79.5 ± 0.26 | 79.0 ± 1.14 | 82.1 ± 1.44 |
| Peroxide Value | 3.3 ± 0.2 | 3.5 ± 0.3 | 3.1 ± 0.66 | 3.9 ± 0.36 |
| Refractive Index | 1.460 ± 0.003 | 1.461 ± 0.002 | 1.461 ± 0.001 | 1.462 ± 0.002 |
| Specific Gravity | 0.928 ± 0.004 | 0.929 ± 0.003 | 0.930 ± 0.001 | 0.930 ± 0.002 |

[a] Each value is a mean of weight of three batches each containing fifty fruits,
[b] Mean of data from at least three determinations

**Table 4** Summary of results from analysis of fruits from plants with yellow or purple flower colours.

| ANALYTICAL DATA OF FRUITS | FRUIT SAMPLES FROM PLANTS WITH | |
|---|---|---|
| | YELLOW FLOWERS | PURPLE FLOWERS |
| % Kernel/Fruit | 35.1 ± 6.1 | 38.1 ± 2.3 |
| Oil Yeild % | 63.3 ± 0.70 | 61.3 ± 0.51 |
| Saponification Value % | 124.3 ± 0.37 | 121.7 ± 0.50 |
| Unsaponification Value | 0.37 ± 0.03 | 0.41 ± 0.07 |
| Free Fatty Acid % | 0.62 ± 0.02 | 0.61 ± 0.05 |
| Iodine Value | 79.4 ± 0.27 | 79.5 ± 0.41 |
| Peroxide Value | 3.8 ± 0.31 | 3.7 ± 0.24 |
| Refractive Index | 1.461 ± 0.001 | 1.461 ± 0.002 |
| Specific Gravity | 0.929 ± 0.002 | 0.928 ± 0.002 |
| Viscosity (Centripose) | 20.11 ± 0.22 | 20.13 ± 0.18 |

Each Value is a mean of data from at least three determinations

**Table 5** Common and major fatty acids of oil from seeds in four different Nigerian geographical zones (number in parentheses represents different samples from the same geographical zone).

| VARIETY | | FATTY ACIDS (%, Weight) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Location | Fruit-type | 14.0 | 16.0 | 16:1 & 7 | 16:0 | 18:1 & 9 | 18:2 & 6 | 18:3 & 3 | Total Saturated | Total Unsaturated |
| Enugu | (1) | 0.31 | 17.72 | 0.10 | 5.22 | 46.02 | - | 0.74 | 23.35 | 46.86 |
| | (2) | 0.37 | 19.33 | 0.35 | 6.02 | 40.38 | 16.57 | 0.70 | 25.72 | 57.98 |
| | (3) | 0.48 | 18.37 | 0.18 | 6.31 | 39.94 | 13.12 | 0.92 | 25.16 | 56.16 |
| | (4) | 0.43 | 20.66 | 0.30 | 7.00 | 36.74 | 8.74 | - | 28.29 | 38.51 |
| | (2)[a] | 0.48 | 21.78 | 0.28 | 7.19 | 45.78 | 14.79 | - | 29.75 | 61.18 |
| | (4)[a] | 0.37 | 23.41 | 0.32 | 6.63 | 44.38 | 11.75 | 0.45 | 30.41 | 56.90 |
| Zaria | (1) | 0.14 | 20.19 | 0.24 | 8.07 | 45.29 | 16.91 | 0.26 | 28.96 | 54.44 |
| | (3) | 0.22 | 20.12 | 0.25 | 7.39 | 47.00 | 14.77 | 0.47 | 27.73 | 62.49 |
| | (4) | 0.38 | 20.21 | 0.28 | 7.63 | 46.00 | 16.00 | 0.49 | 28.22 | 62.77 |
| Edidi | (1) | 0.56 | 14.12 | 0.32 | 5.01 | 54.06 | 13.85 | 1.35 | 19.78 | 51.58 |
| | (2) | 0.17 | 20.00 | 0.29 | 7.76 | 40.85 | 8.85 | 0.12 | 28.22 | 50.11 |
| | (3) | 0.72 | 15.95 | 0.17 | 5.98 | 34.12 | 10.76 | 0.16 | 22.65 | 45.21 |
| | (4) | 0.31 | 19.18 | 0.23 | 6.56 | 36.58 | 7.95 | 1.39 | 26.05 | 46.15 |
| Ilorin | (1) | 0.13 | 20.42 | 0.25 | 7.35 | 46.06 | 12.58 | 0.44 | 28.90 | 58.33 |
| | (2) | 0.17 | 18.34 | 0.21 | 5.28 | 30.44 | 14.05 | 1.33 | 23.79 | 46.03 |
| | (3) | 0.07 | 21.00 | 0.19 | 7.20 | 43.39 | 14.68 | 0.45 | 28.28 | 58.71 |
| | (4) | 0.32 | 15.97 | 0.21 | 5.80 | 33.76 | 16.97 | 0.71 | 22.09 | 51.65 |

[a] Flower with purple colour.

**Table 6** Minor and unusual fatty acids composition of oilseeds from four Nigerian geographical zones (numbers in parenthesis represent different sample from the same geographical zone.

| VARIETY | | FATTY ACIDS (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Location | Fruit-type | 15:0 | 15:1 | 17:0 | 18:1 to 7 | 20:0 | 20:1 to 9 | 22:0 | 24:0 |
| Enugu | (1) | 0.18 | 2.31 | 0.68 | 0.10 | 1.01 | - | 0.98 | 1.47 |
| | (2) | - | - | 0.15 | 0.49 | 1.30 | 0.24 | 0.18 | 0.54 |
| | (3) | 0.17 | - | 0.13 | 0.50 | 1.51 | 0.22 | 0.82 | 0.63 |
| | (4) | - | 0.51 | 0.16 | 0.60 | 1.41 | - | 0.67 | 0.69 |
| | (2[a]) | - | - | 0.13 | 0.45 | 1.40 | 0.28 | 0.88 | 0.56 |
| | (4[a]) | 0.22 | 0.30 | 0.13 | 0.52 | 1.54 | 0.21 | 0.78 | 0.52 |
| Zaria | (1) | - | - | 0.15 | 0.46 | 1.40 | 0.23 | 0.66 | 0.54 |
| | (2) | 0.54 | 0.68 | 0.14 | 0.53 | 1.51 | 0.17 | 0.97 | 0.67 |
| | (3) | - | - | 0.19 | 0.47 | 1.56 | 0.20 | 0.65 | 0.35 |
| | (4) | - | - | 0.16 | 0.51 | 1.45 | 0.12 | 0.64 | 0.27 |
| Edidi | (1) | - | - | 0.12 | 0.14 | 1.07 | 0.78 | 0.57 | 0.41 |
| | (2) | - | - | 0.20 | 0.70 | 1.77 | 0.29 | 0.90 | 0.82 |
| | (3) | - | 0.09 | 0.12 | 0.50 | 1.26 | 0.19 | 1.23 | 0.55 |
| | (4) | - | - | 0.12 | 0.56 | 1.10 | 0.21 | 0.45 | 1.49 |
| Ilorin | (1) | - | - | 0.14 | 0.50 | 1.31 | 0.32 | 0.59 | 1.29 |
| | (2) | - | - | 0.13 | 0.65 | 0.98 | - | 1.13 | 0.88 |
| | (3) | - | - | 0.11 | 0.55 | 1.27 | 0.19 | 0.87 | 0.81 |
| | (4) | - | - | 0.12 | 0.59 | 1.41 | 0.28 | 0.81 | 1.62 |

[a]Flower with orange colour.

The number of kernels per fruit and the oil yield (Table 2a) are significantly different among geographical locations. There is also significant difference in the pattern of variation between weight of fruits and their geographical locations. Statistical analysis of the results from analysis of the fruits and oils from the four locations (Table 3) indicates that there is no significant variation in the value of free fatty acids, peroxide value, refractive index and specific gravity for oils from the four geographical locations. Zaria sample with the lowest weight of fruit has the highest oil yield and vice versa whereas among Zaria, Ilorin and Edidi on one hand, and Enugu, Ilorin and Edidi on the other hand, there is no significant variation.

All data on the oils (Table 4) from plants producing purple flower suggest that there is no distinct varietal classification of the plant based on the flower colour. Statistical analysis of the results of the fatty acid (Tables 5, 6) using Duncan grouping procedure for the degree of unsaturation does not indicate any convincing variation among the seed number and geographical location varieties. The raw data however is considered to provide possible guide to plant breeders that plants in Zaria with the lowest rainfall, have the lowest number of fruits per plant, highest oil content and highest degree of unsaturation for the oils.

Our studies have involved the four-seed-type fruits from each plant stock. The anticipated genetic variation basic to chemotaxonomy is not obvious for the four-seed-types from the data in our present studies. The persistence of the four-seed-type in the four geographical locations suggests genetic heredity that is not influenced by climatic variations. Propagation of *T. peruviana* plant using each seed-type as cultivar is desirable and strongly recommended to plant breeders. The high oil and protein content provides sufficient incentive for propagation of *T. peruviana* plant to improve the economic status as in Sunflower Helianthus and Rapeseed L. Work is in progress on irradiation of both the fruits and the kernels for possible genetic variations that may produce strains with little or no toxins and retain the high protein and oil content. Future studies shall be concerned with determining other forms of propagation, particularly cloning, to produce varieties for specialty utility.

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