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References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

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ARTICLES

Potential to increase cassava yields through cattle manure and fertilizer application: Results from Bunda College, Central Malawi
Leo Mathias and Vernon H Kabambe

Diversity status of the family, Euphorbiaceae in about 30 km radius of Kudankulam Nuclear Power Project area, Southern India
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Potential to increase cassava yields through cattle manure and fertilizer application: Results from Bunda College, Central Malawi

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In Malawi, increasing human population and low productivity of cassava (*Manihot esculenta* [Crantz]), the second most important food crop after maize, necessitate the intensification of agronomic options for cassava production. A study was therefore conducted in 2012-2013 season on a chromic Luvisol at Bunda College (14° 35 S; 33° 50 E'), Central Malawi, to evaluate the effects of inorganic fertilizer (kg ha⁻¹ N : P₂O₅ : K₂O : S at 0 and 46:42:0:8) and cattle manure (0, 5 and 10 t ha⁻¹) on cassava growth and yield in a 3 x 2 factorial randomized complete block design replicated four times. The sweet variety Mbundumali with 9-15 months maturity and potential yield of 25 t/ha was used. The study site’s inherent soil chemical characteristics in the 15-30 cm depth were: P = 102 ppm, K = 3.87 meq 100g⁻¹, N = 0.16%, pH = 5.4, organic matter = 1.79 and N = 0.064. From the results, tuber fresh yield was significantly increased by application of both cattle manure (P = 0.043) and inorganic fertilizer (P = 0.001) while there was no significant manure x inorganic fertilizer interaction. Tuber fresh yield was maximum at 27.6 t/ha with application of cattle manure at 5 t ha⁻¹, however increasing the rate of cattle manure to 10 tha⁻¹ did not increase the tuber yield further. Fresh tuber yield increased from 22.8 to 29.2 t ha⁻¹ with inorganic fertilizer application. Yield without fertility amendments was 21 t ha⁻¹, suggesting that appropriate amendments may raise yields above potential. There were also significant effects of inorganic fertilizer on branches per plant, tubers per plant and tuber length, while manure had no effect on these variables.

**Key words:** Cassava, cattle dung, integrated soil fertility management, cassava growth.

**INTRODUCTION**

Cassava is the most important food crop in Malawi after maize. In 2011-12, cassava was grown on an area of 207,008 ha with an average yield of 22 t ha⁻¹ (MoAFS, 2012). Cassava is also an important cash crop when sold to urban dwellers that use it for domestic consumption. The crop is also an important cash crop for starch production and confectionery. The crop is tolerant to low soil fertility and drought tolerant (Janssens, 2001). Janssens (2001) indicated that cassava requires a minimum of 500 mm rainfall spread over six months.

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Malawi has unimodal rainfall pattern with 5-6 months of rainfall season spanning from November to April/May. Annual rainfall ranges from 600 to 2000 mm depending on agroecological zones (Mviha et al., 2011). Yield potential of cassava ranges from 15-25 t ha⁻¹ for sweet varieties and 25-40 for bitter varieties (Mviha et al., 2011; MoAFS, 2012). There are number of constraints to cassava production, including diseases, insect pests, unavailability of quality planting material, and short rainfall periods (MoAFS, 2012). Cassava is well adapted to poor or degraded soils due to tolerance to low pH, high exchangeable Al and low concentration of P in the soil solution (CIAT, 1978; Howeler, 1991; Janssens, 2001; Howeler, 2002). When planted in natural soils, cassava’s fibrous roots become infected with native soil mycorrhiza. The resulting hyphae grow into the surrounding soil and help in the uptake and transport of P to the cassava roots. Although, cassava tolerates drought and low soil fertility, maximum yields are only possible with adequate amount and duration and soil fertility. In Malawi, there are no recommendations for inorganic fertilizer applications for cassava production (MoAFS, 2005). However, poor soil fertility is a growing constraint in crop production in Malawi (Kumwenda et al., 1997; Snapp, 1998; ICRISAT/MAI, 2000; Kanyama-Phiri et al., 2000; Ngwira et al., 2013). The continued cropping of cassava without fertilizer application can result in soil nutrient depletion. Howeler et al. (1990) reported that 4.5, 0.83 and 6.6 kg mineral N P and K are removed per tonne of dry tuber yield. Howeler (2002) recommended that 60 kg N, 10-20 kg P₂O₅, and 50 kg K₂O ha⁻¹ should be applied to the soil for an expected yield of 15 t/ha where all stems and leaves are returned to the soil. Also, Asare et al. (2009) noted that cassava is known to respond to application of organic and inorganic fertilizers. Several other reports indicate that the crop is responsive to fertilizer use (FAO, 1994; Kamaraj et al., 2008; Adjei-Nsiah and Issaka, 2013). Over-application of N fertilizer may, however lead to unusually luxuriant vegetative growth at the expense of roots and tubers (Vijayan et al., 1969).

One possible reason for lack of response to fertilizer application in Malawi are the relatively short rains of 4-5 months as compared to 9-12 months maturity period, which result in fertilizer not being available to the plants due to dry soil conditions. Application of organic source of nutrients was considered one way to enhance crop response to nutrient applications. Organic matter improves soil tilth, increases water holding capacity, lessens erosion, improves soil aeration and has a beneficial effect on soil microorganisms (Howeler, 1986, 2008). The objective of this study was therefore to explore yield and growth response of mbundumali cassava variety to cattle manure and inorganic fertilizer application.

### MATERIALS AND METHODS

#### Site description, experimental design and treatments

An experiment to explore the role of inorganic fertilizer and cattle manure was conducted on a chromic Luvisol at Bunda Campus of the Lilongwe University of Agriculture and Natural Resources Lilongwe, Malawi in the 2012/13 growing season. The site is 1158 m above sea level, latitude 14° 35’ S and longitude 33° 50’ E. Soil type varies from clay loam to sandy loam textural classes with medium fertility. Mean annual rainfall is approximately 1031 mm with coefficient of variation (cv %) of 16.6% indicating adequate reliability of total rainfall (Jones and Kanyama, 1975). A composite soil sample was taken from the experimental site and analyzed for selected physical texture and chemical characteristics. The results are shown in Table 1. According to Howeler (2002), the following levels are considered as medium for cassava classification: Phosphorus 4-15 ppm, K 0.15 - 0.25 meq/100 g and organic matter (OM) 2-4%. The test site was therefore low in OM for cassava production.

The study was a 3 x 2 factorial field experiment in a randomized complete block design with four replications. The first factor was cattle manure applied at 0, 5 and 10 tonne ha⁻¹ and the second was inorganic fertilizer at 0 and 200 kg ha⁻¹ of the fertilizer compound 23:21:0:4S giving total of 46:42:8 kg ha⁻¹ of N, P₂O₅ and S, respectively. The manure sample was analyzed for N, P, K and % organic matter. The results and amounts of N, P and K applied at 5 and 10 t/ha are shown in Table 2.

#### Plot sizes and field operations

The gross plots comprised of 5 ridges, 7.2 m long spaced at 0.90

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**Table 1.** Chemical and physical property of the soil at the site of experiment.

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Top soil (0-15 cm)</th>
<th>Subsoil (15-30 cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (%)</td>
<td>0.16</td>
<td>0.064</td>
</tr>
<tr>
<td>Phosphorus (ppm)</td>
<td>105.67</td>
<td>12.34</td>
</tr>
<tr>
<td>Potassium (me/100 g)</td>
<td>10.83</td>
<td>3.87</td>
</tr>
<tr>
<td>Soil pH</td>
<td>5.8</td>
<td>5.4</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>1.66</td>
<td>1.79</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>43</td>
<td>34</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>22</td>
<td>25</td>
</tr>
</tbody>
</table>
m. Each net plot comprised of 3 middle ridges of 5.7 m long. Cassava cuttings of 20-30 cm length, with 6-8 nodes per cutting were planted at 0.9 m x 1 plant. The land preparation was done using hoes in November, 2012 while planting was done on 28 December 2012 following adequate rains. Planting materials were obtained from Bunda College Crop and Soil Sciences Students Farm and were 13 months old. The cuttings were disease free from predominantly bacterial bright and cassava mosaic diseases. Cuttings were taken from middle part of the cassava, and were planted on the day of preparation according to recommendations (MoAFS, 2005). The variety used was Mbundumali, an early maturing sweet cassava which matures in 9-15 months and is tolerant to cassava green mite (CGM) and cassava brown streak disease (CBSD) though susceptible to cassava mosaic disease (CMD). It has a yield potential of 15-25 t ha$^{-1}$.

Inorganic fertilizer (NPK) and cattle manure were applied during planting on December 28, 2012 according to earlier treatment descriptions. Banding method was used in application of the cattle manure. Banding inorganic fertilizer, whereby drills were made on the ridges and the manure and/or inorganic fertilizer were applied and covered with soil immediately. However, (2002) reported that optimal yields of cassava were recorded when all fertilizers are either applied at time of planting or at once. Plants were drenched with Dursban on 27 January 2013 for termite control. The plots were hoe-weeded up to 14th week after planting to keep weed competition negligible. Later hand weeding was done due to canopy closure. Harvesting of the cassava was done at 11 months after planting using hoes and shovels to avoid damaging the cassava tubers.

**Data collection and analysis**

Data was collected on daily rainfall, spraying rate at 15 days after planting while stand count was taken at harvest. Plant height (base to the tallest apical leaf) was recorded at 6, 16 and 40 weeks after planting (WAP) as a means of monitoring treatments effects on early and final growth. Five plants were randomly selected from the net plot for plant height for measurements using a ruler (100 cm). Canopy diameter was measured from the same plants at 6 and 16 WAP. At harvest, number of branches and number of stems were determined by counting branches and the stems of the same 5 plants from each net plot. Tuber diameter and tuber length were taken by measuring the cross section of cassava tubers of 5 randomly selected of the representative plants immediately after harvesting. Yield of the cassava was determined by fresh weight, which was done by weighing all the tubers from the net plot and expressed as kg/ha. Harvest index was determined by dividing economic yield (cassava tubers) by total biological yield comprising of both the above ground parts and cassava tubers.

After determining yield data, a random sample of tubers were taken from the 5 plants from the net plot and chopped into smaller pieces. These pieces were mixed and 400 g taken and oven dried at 72°C for 72 h (Koide et al., 2000). The weight after constant value was recorded and dry matter content calculated as percentage. All data were subjected to the analysis of variance procedure using Genstat Release 16. Regression analysis yield and yield components and plant height were also done.

### RESULTS AND DISCUSSION

#### Daily rainfall, sprouting rate (15 days after planting) and final stand count per plot at harvest.

The cumulative daily rainfall for the site is shown in Figure 1. The rainfall total and distribution were considered normal for the area. There were no significant differences on spraying rate at 15 DAP (mean 94.5%) and establishment at harvest (mean 23.0%), such that further differences could be attributed to treatment effects. This early sprouting concurs with good early rainfall recorded (Figure 1). Lebot (2009) indicated that spraying in cassava occurs in first 5-15 days after planting.

#### Manure and inorganic fertilizer effects on tuber fresh yield and yield components

Analysis of variance results showed significant (P <0.05) inorganic manure effects on fresh tuber yield, branches per plant, tubers per plant, tuber diameter, and length (Table 3), but not tuber dry matter % (mean 42%) and harvest index (mean 0.77). Cattle manure application, on the other hand, significantly affected tuber fresh yield only (Table 4). There was no significant manure x inorganic fertilizer effects detected on all these variables.

Inorganic fertilizer increased the cassava fresh yield from 21.89 to 29.16 kg ha$^{-1}$ (Table 3). Results are in agreement with the finding of other researchers (Theodor, 1965; Iman et al. 2013). Zhang et al. (1998) reported that combination of manure and inorganic fertilizers, or of inorganic fertilizers alone, generally resulted in yields of 20-40 t ha$^{-1}$ yield than the application of only organic manures.

Application of 5 t ha$^{-1}$ cattle manure significantly increased tuber fresh yield from 21.90 to 27.61 kg ha$^{-1}$ and there was no further increase with 10 t/ha of cattle manure (Table 4). Results are in agreement with Rammachat et al. (2001) who reported that application of animal manure only increased the root fresh yield of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Analytical composition (%)</th>
<th>Quantity of CM applied (5 t/ha)</th>
<th>Quantity of CM applied (10 t/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (N)</td>
<td>0.99</td>
<td>49.5</td>
<td>99</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>0.33</td>
<td>16.3</td>
<td>33</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>1.45</td>
<td>72.5</td>
<td>145</td>
</tr>
<tr>
<td>Organic matter</td>
<td>4.741</td>
<td>237.5</td>
<td>474.1</td>
</tr>
</tbody>
</table>

**Table 2. Chemical composition of the cattle manure and quantities applied.**
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Figure 1. Cumulative daily rainfall, Bunda College 2012-13 season.

Table 3. Effects of inorganic fertilizer application on cassava fresh tuber yield, number of tubers per plant, stem weight, tuber diameter (TD), tuber length (TL) and branches/plant.

<table>
<thead>
<tr>
<th>Fertilizer (kg/ha) N:P₂O₅:K₂O:S</th>
<th>Fresh yield (t ha⁻¹)</th>
<th>No of tubers/plant</th>
<th>Stem wt (t ha⁻¹)</th>
<th>TD (cm)</th>
<th>TL (cm)</th>
<th>No. of branches/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21.89</td>
<td>10.0</td>
<td>9.96</td>
<td>3.601</td>
<td>32.82</td>
<td>6.27</td>
</tr>
<tr>
<td>46:41:8</td>
<td>29.16</td>
<td>12.5</td>
<td>12.45</td>
<td>3.987</td>
<td>38.89</td>
<td>8.26</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>3.94</td>
<td>2.2</td>
<td>2.35</td>
<td>0.35</td>
<td>3.9</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 4. Effects of cattle manure application on cassava tuber yield (t ha⁻¹).

<table>
<thead>
<tr>
<th>Manure rate (t ha⁻¹)</th>
<th>Fresh tuber yield (kg ha⁻¹)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21.90ᵃ</td>
</tr>
<tr>
<td>5</td>
<td>27.61ᵇ</td>
</tr>
<tr>
<td>10</td>
<td>27.08ᵇ</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>4.83</td>
</tr>
</tbody>
</table>

*Means in column with the same letter are not significantly different at P=0.05%.

cassava. Similar responses to inorganic fertilizer application have been reported widely (FAO, 1994; Kamaraj et al., 2008; Asare et al., 2009; Adjei-Nsiah and Issaka, 2013). The manure analysis showed that cattle manure had high organic matter content (4.7%) and also supplied 49 and 99 kg ha⁻¹ N at the 5 and 10 t/ha. Thus, the response to manure is expected particularly noting that the organic matter and N content of the site was low (Table 2).

Asare et al. (2009) reported a response of cassava to N fertilizer only. Kamaraj et al. (2008) reported positive response to fertilizer with rates of 60–90 kg/ha for N, 30-90 kg/ha for P, and 80-160 kg/ha for K, with yields of up to 52 t/ha. Organic matter works as slow release source of nutrients and prevents leaching losses. The fresh tuber yield produced by both manure and inorganic fertilizer only are greater than the estimated fresh yield under smallholder farmers in Malawi which falls within the ranges of 8,000 to 20,000 kg/ha (MoAFS, 2012). The manure rate of 5 t/ha was relatively low as compared to recommended rate of 12.5 t/ha in maize (MoAFS, 2012), and perhaps has fair chance for adoption if widely verified. The cassava fresh yields produced by application of cattle manure only were higher than the potential yield of 15-25 t/ha for the Mbundumali variety (MoAFS, 2012) which was used in the study. Adjei-Nsiah and Issaka (2013) reported average fresh tuber yield increase from 13.7 t ha⁻¹ without amendment to 23.7 t ha⁻¹.
Table 5. Effect of inorganic fertilizer application on base stem diameter, canopy width and plant height at different sampling times.

<table>
<thead>
<tr>
<th>Inorganic fertilizer rate kg ha(^{-1})</th>
<th>Growth parameter, and time of recording, weeks after planting (WAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Canopy width, cm</td>
</tr>
<tr>
<td>Fertilizer kg/ha (N):P(2):O(3):K(2):S(6)</td>
<td>6 WAP</td>
</tr>
<tr>
<td>0</td>
<td>30.4</td>
</tr>
<tr>
<td>46:41:8</td>
<td>39.1</td>
</tr>
<tr>
<td>F prob</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 6. Effect of inorganic fertilizer application on base stem diameter, canopy width and plant height at different sampling times, weeks after planting, WAP.

<table>
<thead>
<tr>
<th>Cattle manure rate</th>
<th>Growth parameter and time of recording, weeks after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Canopy width, cm</td>
</tr>
<tr>
<td></td>
<td>6 WAP*</td>
</tr>
<tr>
<td>0</td>
<td>45.3a</td>
</tr>
<tr>
<td>5 t/h</td>
<td>55.7b</td>
</tr>
<tr>
<td>10</td>
<td>55.4b</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>9.4</td>
</tr>
</tbody>
</table>

*Means in column with the same letter are not significantly different at \(P=0.05\%\).

with application of 4 t ha\(^{-1}\) poultry manure. Cassava response to manure was also reported by Odedina et al. (2011) and Ojeniyi et al. (2012).

The significant increase in tubers/plant, stem weight, tuber diameter and length supports the yield increased observed. An increase in number of branches per plant is important to expose the cassava leaves to sunlight for photosynthesis and increased translocation for higher photosynthate accumulation (Okogun et al., 1999). Increased branching may also suppress weeds. Increased tuber diameter and length is important for marketing purposes. IITA/SARRNET (2007) reported that the fresh cassava market is the largest marketing channel in Malawi. Medium to large tuber sizes are preferred for buyers who eat it raw or boiled at home because the tubers are easier to peel and can be chopped into desired sizes. Traders avoid unattractive small tubers to optimize transport.

Manure and inorganic fertilizer effects on cassava growth

Inorganic fertilizer and manure main effects on growth parameters of stem diameter, canopy width and plant height are shown in Tables 5 and 6. Canopy diameter of 90 cm reflects full canopy closure, as plants were planted in rows 90 cm apart and between plants. It is interesting to note that significant differences were observed at 6 WAP for both sources, suggesting importance of both fertility amendments in improving crop growth. Canopy diameter in cassava ensures large surface solar interception and photosynthesis (Lebot, 2009). Plants are thus more likely to suppress weeds. However, excessive foliage may lead to shedding and other leaves serving as net users of photosynthates. Tolessa (2001) reported that after 120 DAP, the leaves are able to intercept most of the radiation falling on the canopy and it is the time when the maximum size of the canopy with the maximum dry matter partition of the leaves and stems are obtained. Howeler (1990) further stated that large bulk of foliage are created by the action of nitrogen and consequently an extensive assimilating area, a prerequisite for the good development of the tubers. Plant height affects cassava growth yield similarly.

Regression relationship between growth variables, yield components versus tube fresh yield

Regression analysis was conducted on growth parameters, yield components versus yield. Significant positive regression was found between plant height at 8, 16 and 40 WAP, stem weight, tubers per plant and yield (Table 7), but not with stems per plant. The results suggest that the growth responses recorded accounted for yield differences detected. It is of interest to note significant relationship for plant height vs. yield at an 8 weeks after planting, as this indicates that good early growth is important for yield, confirming the role of fertilizer in increasing yield. The importance of the other parameters to yield has been discussed earlier.
Table 7. Regression relationship between plant height (PHT, cm) stem weight (STMWT, t ha\(^{-1}\)), stems per plant (STEMPLT) and tubers per plant (TUBPLANT) fresh tuber yield (Y = tones ha\(^{-1}\), TUBERHA).

<table>
<thead>
<tr>
<th>Pair of variables</th>
<th>Equation</th>
<th>Significance for intercept</th>
<th>Significance for regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHT at 40 WAP vs. TUBERHA</td>
<td>Y = -6.57 + 0.197X</td>
<td>t pr = 0.43</td>
<td>F Pr &lt;0.001</td>
</tr>
<tr>
<td>PHT at 16 WAP vs. TUBERHA</td>
<td>Y = 6.77 + 0.21X</td>
<td>t pr = 0.29</td>
<td>F Pr =0.001</td>
</tr>
<tr>
<td>PHT at 8 WAP vs TUBERHA</td>
<td>Y = 15.6 + 0.22X</td>
<td>t pr = 0.001</td>
<td>F Pr 0.024</td>
</tr>
<tr>
<td>STMWT vs. tuberha</td>
<td>Y=16.3 + 0.823X</td>
<td>t pr = &lt;001</td>
<td>F pr 0.036</td>
</tr>
<tr>
<td>STMPLT vs. tuberha</td>
<td>Y = 18.8 + 2.23</td>
<td>t pr = 0.002</td>
<td>F Pr 0.208</td>
</tr>
<tr>
<td>TUBPLANT vs. tuberha</td>
<td>Y = 13.5 +1.06</td>
<td>t pr = 0.007</td>
<td>F Pr 0.012</td>
</tr>
</tbody>
</table>

DISCUSSION

The observed cassava yield and growth responses to organic and inorganic are interesting considering the fairly high fertility status of the soil. The soil P and K were high while N was low according to Howeler (2002). Once complete ground cover is reached, cassava shades out weeds (Pellet et al., 1997). The crop was observed for diseases incidence and there was none observed, such that the responses to fertilizer are not confounded disease stress. Anneke et al. (2010) reported that with fertilizer use, the canopy closes within approximately 3 MAP giving potential for weed suppression. Canopy closure also helps to reduce water runoff, consequently reducing soil erosion (Zhang et al., 1998). Increase in growth traits by either the inorganic fertilizer or manure are likely to have contributed to the increased tuber fresh yield in the inorganic fertilizer and manure treatments. Considering that the status of P and K for the soils was high, the responses are likely due to N application. Inorganic fertilizer, although applied at the rate of only 23 kg/ha gave significant increase on cassava tuber yield, suggesting importance of nitrogen. Organic fertilizer has many benefits over inorganic fertilizer. Manure can be locally available for some farmers and it helps to improve the soil structure for easy penetration of shoots and development of the roots. Nutrients contained in organic manures are released more slowly and are stored for a longer time in the soil, thereby ensuring a long residual effect (Tisdale et al., 1985). The yields of 27 tha\(^{-1}\) for treatments with fertility amendments were above the potential yield for the variety, indicative of potential to raise the yield potential for cassava. The positive regression relationships between growth parameters and yield components (except stems per plant) (Table 6) suggest that yield responses are a result of better growth and possible photosynthetic capture. Many reports show that fertility status of smallholder farmers in Malawi is much poorer than the study site (Snapp, 1988; Kabambe et al., 2012; Ngwira et al., 2012), thus suggesting that similar responses would be obtained on farmers’ fields. These results therefore justify extensive studies to develop fertilizer responsive guides for different soil types and agro-ecological zones.

Conflict of interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

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Short Communication

Diversity status of the family, Euphorbiaceae in about 30 km radius of Kudankulam Nuclear Power Project area, Southern India

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A detailed Euphorbiaceae flora study was carried out from 30 km radius of Kudankulam Nuclear Power Project area. The survey was conducted from September 2012 to August 2013. A total of 32 species under 12 genera belonging to the family Euphorbiaceae were collected and identified. For each species, botanical name, local name, life-form, habitat and flowering season were mentioned. A statistical summary is presented at the end of the checklist. The area shows a high diversity in its terrestrial flora and has an important role in the conservation of biodiversity of the region.

Key words: Euphorbiaceae, life-form, conservation, Nuclear Power Project, Kudankulam.

INTRODUCTION

Euphorbiaceae is composed of 334 genera (Webster, 1994) and over 8,000 species (Radcliffe Smith, 2001) as shown in some previous work worldwide (Govaerts et al., 2000; Balakrishnan and Chakrabarty, 2007). The earliest floristic exploration of the Tirunelveli and Kanyakumari region floras was undertaken in the last century (Beddome, 1877; Lawson, 1894). Most of the existing studies in the Agastiyamalai region pertain to the floristic enumerations from the Kanyakumari, Tirunelveli (Lawrence, 1959, 1960; Nayar, 1959; Sankaranarayanan, 1960; Subramanyam and Henry, 1973; Rao et al., 1974; Sharma et al., 1973). Vanila (2003) collected 793 species from 416 genera belonging to 102 families. Uthayakumari Kalavathy (2004) recorded 412 species of monocotyledons belonging to 185 genera and 26 families from Tirunelveli hills and Jothi (2001) described the Euphorbiaceae floristic enumerations from the Tirunelveli hills. Biological diversity is now increasingly recognized as a vital parameter to assess global and local environmental changes and sustainability of developmental actives. Summarily, the study aims to provide the taxonomic diversity of Euphorbiaceae as part of the environment impact assessment studies undertaken on biodiversity around Kudankulam Nuclear Power Plant area in Radhapuram taluk of Tirunelveli.
Table 1. Diversity status of Euphorbiaceous plants in KKNPP site.

<table>
<thead>
<tr>
<th>Genera and species</th>
<th>Local name</th>
<th>Habitat</th>
<th>Flowering season</th>
<th>Status</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acalypha amentacea Roxb.</td>
<td>S</td>
<td>Throughout the year</td>
<td>Rare</td>
<td>SJ</td>
<td></td>
</tr>
<tr>
<td>Acalypha ciliata Forssk.</td>
<td>H</td>
<td>September to January</td>
<td>Frequent</td>
<td>SJ</td>
<td></td>
</tr>
<tr>
<td>Acalypha hispida Burm.f.</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>Acalypha indica L.</td>
<td>Kuppaimeni</td>
<td>H</td>
<td>July to March</td>
<td>Common</td>
<td>P, W</td>
</tr>
<tr>
<td>Acalypha paniculata Miq.</td>
<td>H</td>
<td>Throughout the year</td>
<td>Common</td>
<td>W</td>
<td></td>
</tr>
<tr>
<td>Acalypha wilkesiana Müll.Arg.</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>Breynia retusa (Dennst.) Alston</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>SJ</td>
<td></td>
</tr>
<tr>
<td>Codiaeum variegatum (L.) Juss.</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>Croton bonplandianus Baill.</td>
<td>Mannannai chedi</td>
<td>H</td>
<td>Throughout the year</td>
<td>Common</td>
<td>P</td>
</tr>
<tr>
<td>Euphorbia antiquorum L.</td>
<td>Sadarakalli</td>
<td>S</td>
<td>January to April</td>
<td>Common</td>
<td>P, SJ</td>
</tr>
<tr>
<td>Euphorbia heterophylla L.</td>
<td>Paal perukki</td>
<td>H</td>
<td>October to March</td>
<td>Frequent</td>
<td>CF</td>
</tr>
<tr>
<td>Euphorbia heyneana subsp. galioides (Boiss.) Panigrahi</td>
<td></td>
<td>H</td>
<td>Throughout the year</td>
<td>Rare</td>
<td>SJ</td>
</tr>
<tr>
<td>Euphorbia hirta L.</td>
<td>Ammanpatchaiarisi</td>
<td>H</td>
<td>Throughout the year</td>
<td>Frequent</td>
<td>CF</td>
</tr>
<tr>
<td>Euphorbia ingens E.Mey. ex Boiss.</td>
<td>S</td>
<td>Throughout the year</td>
<td>Frequent</td>
<td>P, SJ</td>
<td></td>
</tr>
<tr>
<td>Euphorbia rosea Retz.</td>
<td>H</td>
<td>Throughout the year</td>
<td>Common</td>
<td>SJ</td>
<td></td>
</tr>
<tr>
<td>Euphorbia thymifolia L.</td>
<td>Sitrupaladai</td>
<td>H</td>
<td>Throughout the year</td>
<td>Common</td>
<td>SD</td>
</tr>
<tr>
<td>Euphorbia tirucalli L.</td>
<td>Thirukalli</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>SJ</td>
</tr>
<tr>
<td>Euphorbia tithymaloideis L.</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>Euphorbia tortilis Rottler ex Ainslie</td>
<td>Thirukalli</td>
<td>S</td>
<td>January to February</td>
<td>Common</td>
<td>SJ</td>
</tr>
<tr>
<td>Euphorbia umbellata (Pax) Bruyn</td>
<td>S</td>
<td>August to March</td>
<td>Common</td>
<td>P, SJ</td>
<td></td>
</tr>
<tr>
<td>Flueggea leucopryrus Willd.</td>
<td>Pulachi</td>
<td>S</td>
<td>June to November</td>
<td>Common</td>
<td>P, SJ</td>
</tr>
<tr>
<td>Hevea brasiliensis (Willd. ex A.Juss.) Müll.Arg.</td>
<td>Rupper maram</td>
<td>T</td>
<td>Throughout the year</td>
<td>Common</td>
<td>C</td>
</tr>
<tr>
<td>Jatropha curcas L.</td>
<td>Kattuamanaku</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>P, SJ</td>
</tr>
<tr>
<td>Jatropha glandulifera Roxb.</td>
<td>Veilai</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>W</td>
</tr>
<tr>
<td>Jatropha gossypiiifolia L.</td>
<td>Kattuamanaku</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>P, SJ</td>
</tr>
<tr>
<td>Jatropha integerrima Jacq.</td>
<td>S</td>
<td>Throughout the year</td>
<td>Rare</td>
<td>SJ</td>
<td></td>
</tr>
<tr>
<td>Jatropha maheswarii Subram. &amp; Nayar</td>
<td>Athalai</td>
<td>S</td>
<td>December to January</td>
<td>Endemic</td>
<td>S, P, SJ</td>
</tr>
<tr>
<td>Jatropha multifida L.</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>Manihot esculenta Crantz</td>
<td>Mara valli</td>
<td>S</td>
<td>Throughout the year</td>
<td>Common</td>
<td>C</td>
</tr>
<tr>
<td>Microstachys chamaelea (L.) Müll.Arg.</td>
<td>H</td>
<td>Throughout the year</td>
<td>Common</td>
<td>SJ, W</td>
<td></td>
</tr>
<tr>
<td>Ricinus communis L.</td>
<td>Amanakku</td>
<td>S</td>
<td>October to March</td>
<td>Common</td>
<td>C</td>
</tr>
<tr>
<td>Tragia involucrata L. var. involucrata</td>
<td>H</td>
<td>Throughout the year</td>
<td>Common</td>
<td>P, SJ</td>
<td></td>
</tr>
</tbody>
</table>

Sandy (S), wetland (W), scrub jungle (SJ), cultivated field (CF), cultivated (C), sand dune (SD), plains (P) and ornamental (O).

district, Tamil Nadu.

MATERIALS AND METHODS

Study area

The site for Kudankulam Nuclear Power Project (KKNPP) is situated in Kudankulam Village, Tirunelveli district, Tamil Nadu, India. The site is on the shore of the Gulf of Mannar and is located near the South eastern tip of India. The study area covered is 30 km radius of KKNPP. The study area lies between latitudes 8° 28' to 8° 28' North and longitudes 77° 28' to 77° 57' of East in the terrestrial region including fresh water and wetlands. The nearest village to the site is Kudankulam and Idinthakarai. The study area covered Radhapuram in Tirunelveli district, Agasteeswaram and Thovalai taluks in Kanyakumari district, Tamil Nadu. The east of the KKNPP is lined up by the seashore of Gulf of Mannar and the north, west and south are bound by land (Tirunelveli district) (Table 1).

The Plant Site Boundary Wall Layout of the site area is presented in Figure 1. The common habitats of terrestrial, hydrophytes and marshy vegetation are in the plains, coastal dune, scrub jungle, dry deciduous forest, ponds, tanks, rivers, canal banks, ditches, rice fields and low-lying waterlogged areas are ideal habitats for many terrestrial, aquatic, semi aquatic wetland and marsh plants.

Method

Frequent field surveys were carried out in about 30 radius of Kudankulam Nuclear Power Project during September 2012 to August 2013. Diversity of each species and their distribution in Gulf
of Mannar Biosphere Reserve was determined by the Flora of Gulf of Mannar Biosphere Reserve (Daniel and Umamaeswari, 2001). The endemic and rare status of plant was determined using the published flora of Daniel and Umamaeswari (2001). The entire
Results and Discussion

Euphorbiaceae is the third largest family with 32 species of plants belonging to 12 genera and considered as one of the best represented families of about 30 km radius of Kudankulam Nuclear Power Project area.

The earliest floristic exploration of the Tirunelveli and Kanyakumari region floras was undertaken in the last century (Beddome, 1877; Lawson, 1894). Most of the existing studies in the Agastyamalai region pertain to the floristic enumerations from the Kanniakumari, Tirunelveli (Lawrence, 1959; 1960; Nayar, 1959; Sankaranarayanan, 1960; Subramanayam and Henry, 1973; Sharma et al., 1973; Rao et al., 1975). Vanila (2003) collected 793 species from 416 genera belonging to 102 families. Uthayakumari Kalavathy (2004) recorded 412 species of monocotyledons belonging to 185 genera and 26 families from Tirunelveli hills. But, Jothi (2001) described the Euphorbiaceae floristic enumerations from the Tirunelveli hills and also intraspecific variation in some species of Euphorbiaceae from Tirunelveli hills (Jothi and Manickam, 2005). Ayyanar and Ignacimuthu (2010) studied diversity, conservation status and medicinal plants of the family euphorbiaceae in Tirunelveli hills, Jatropha maheswarii Subr. & Nayar is found in Gulf of Mannar Biosphere Reserve and identified as strict endemic plants (Daniel and Umamaeswarei, 2001).

Conclusion

KKNPP area has great diversity of plants with varied economic importance and most of the species are locally threatened (Table 1). Sand quarrying in major parts of the coastal dune area has resulted in geomorphological changes only to the disadvantage of the people around there. This has become a social problem leading to clash between communities in the mainland coast where there is sand mining for quartz. Some of the threats like heavy influence of plain land converted for housing development, construction activities adversely affected the existing ecosystem. J. maheswari Subr. & Nayar is one of the important medicinal floras. This species should be conservation need.

Conflict of Interest

The authors did not declare any conflict of interest.

Acknowledgement

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Beddome RH (1877). The forest and flora of Tinnevelly district. The Indian Forester 3:19-25.
Pollen morphology of six species of subfamily Stachyoideae (Lamiaceae) in Saudi Arabia

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The pollen morphological characters of 6 species belonging to 4 genera of the subfamily Stachyoideae (Lamiaceae) growing naturally in Saudi Arabia were investigated with the aid of light microscope (LM) and scanning electron microscope (SEM), to find new features that might increase knowledge of pollen morphology of the species, and also to help the taxonomic characterization of the Stachyoideae genera. The morphological characters studied were size, shape, tectum surface ornamentation, number and type of the colpi. The study indicated that the average size of the pollen was different among the species since the smallest size was that of Nepeta deflersiana (P = 25.2 ± 2 and E = 15 ± 3) while the largest size was that of Salvia aegyptiaca (P = 36.5 ± 2 and E = 30.3 ± 2). Pollen shape is spheroidal to sub-spheroidal or prolate. The fine structure of the exine of pollen was slightly different among investigated species. The number and type of colpi of the pollen in species studied were 6-zonocolpate type except those of N. deflersiana and Otostegia fruticosa ssp. schimperi having 3-zonocolpate.

Key words: Pollen grains, pollen morphology, Lamiaceae, Stachyoideae, Saudi Arabia.

INTRODUCTION

The family Lamiaceae is divided into several subfamilies including the subfamily Stachyoideae which is represented by 15 species in Saudi Arabia (Al Watban, 2004). Contribution to the pollen morphology of some members of the Lamiaceae has been studied by Erdtman (1945), Emboden (1965), Belkin (1972), Mukherjee (1972) and Abu-Asab and Cantino (1992, 1994), trying to use the pollen morphology in classification of Lamiaceae members. However, morphological study on pollen grains of the tribe Ocimeae was done by Harley et al. (1992) who revealed some differences in their sizes, shapes and exine sculpture, and reported four types of pollen grains. Recently, Perveen et al. (2003) mentioned that the pollen morphology of the Family Lamiaceae from Pakistan does not confirm sub-family level of classification while it may be used in identification of species. Moon et al. (2008) in their study on Tribe Mentheae suggested that the variation in exine ornamentation of the pollen may have systematic value especially at generic level. Also, a study on two Salvia species by Hamzaoglu et al. (2005)
revealed that the pollen shape and its exine ornamentation exhibit obvious variations and could be used at species level. Moreover, Jafari and Nikian (2008) reported that pollen characters of four desert species of *Salvia* may be used for their identification. In another study carried out by Celenk et al. (2008a, b), the pollen characters of the genera *Mentha* L. and *Napata* L. were valuable and may be useful for classification at subgeneric level. Kahraman et al. (2009 a, b, 2010 a, b) and Kahraman and Doghan (2010) reported that the pollen size, shape and exine ornamentation in the genus *Salvia* are important in distinguishing between the species. Dereboylu et al. (2010) in their work on *Salvia willeana* and *Salvia veneris* reported that pollens of the two species were similar in their morphological characters where they are with 6 zonocolpate, subprolate shape, and semi-tectate structure and exhibited bireticulate sculpturing, which cannot be used to distinguish between the two species. Özler et al. (2013) in their study on *Hymenosphae* and *Aethiopsis* sections of the genus *Salvia* found that pollen features of closely related species indicate some differences which can be used for their identification. The previous investigations indicate that there is no final agreement for which level of classification can be useful in using pollen morphological characters in Lamiaceae. The aim of this work was to study pollen morphology of 6 species under 4 genera of the subfamily Stachyioideae, to show some pollen features of these arid species that may increase knowledge of pollen morphology of species and also to help the taxonomic characterization of these 4 genera, since there is no available information on the pollen morphological features of Lamiaceae species which are growing naturally in Saudi Arabia.

**MATERIALS AND METHODS**

The studied pollen materials were obtained from preserved samples in the Ministry of Agriculture and Water, Riyadh, Saudi Arabia. Processing of pollen grains for light microscope (LM) was done using the usual acetolysis method (Punt, 1962). For scanning electron microscope (SEM) studies, pollen grains samples were run through an alcohol series 50, 70, 80, 95 and 100% on the stubs using micro-pipettes, (without losing any pollen). The stubs were coated with gold. The representative pollen grains were photographed at various magnifications in a JSM-5800 LV (JOEL) scanning electron microscope. The measurements were based on 15 readings from each specimen. Descriptive terms were according to Moore and Webb (1978) and Punt et al. (2007). Size measurements for the pollen grains were taken according to Erdtman (1971) (very small < 10 µm in dimensions; small 10-25 µm; medium (25-50) µm; large (50-100) µm; very large (100-200) µm; huge > 200 µm).

**RESULTS**

**Mentha microphylla Koch.**

Most of the pollen grains have yellow to brown colour, small to medium size with sub-spheroidal shape in outline; exine surface is reticulate, perforated to granulate, with 6 colpi, zonocolpate with acute apices. The polar length is 26.8 ± 1 µm and equatorial length, 21.87 ± 2 µm (Figure 1: 1 and Table 1).

**Nepeta deflersiana Schweinf.**

The pollen grains are small triangular shape with yellow colour. Exine surface is reticulate, perforate having distinguished 3 colpi, zonocolpate with acute apices. The polar length, 25.5 ± 2 µm, and equatorial length, 15 ± 3 µm (Figure 1: 2 and Table 1).

**Otostegia fruticosa subsp. schimperi (Boiss.) Tackh.**

The pollen grains have yellow colour, medium size, spheroidal shaped in outline. Pollen with 3 distinctive zonocolpate, exine with perforated surface structure. The polar length, 31.5 ± 2 µm, and equatorial length, 28.6 ± 2 µm (Figure 1: 3 and Table 1).

**Salvia aegyptiaca L.**

Pollen grains are yellow to brown colour, medium size, with sub-spheroidal shaped in outline. Pollen with 6 zonocolpate, exine surface structure is bireticulate-perforate. The polar length, 36.3 ± 2 µm, and equatorial length 30.3 ± 2 µm (Figure 1: 4 and Table 1).

**Salvia deserti Decne.**

Pollen grains are yellow colour, small to medium size with prolate shaped in outline. Pollen with 6 zonocolpate, surface structure of the exine is bireticulate-perforate. The polar length, 36.5 ± 2 µm, and equatorial length, 22 ± 2 µm (Figure 1: 5 and Table 1).

**Salvia spinosa L.**

The pollen grains are yellow to brown in colour, medium size and circular to ovate shaped in outline (under LM). Pollen with 6 zonocolpate, the exine is bireticulate-perforate. The polar length, 34.3 ± 2 µm, and equatorial length 27.5 ± 3 µm (Figure 1: 6 and Table 1).

**DISCUSSION**

The pollen morphological characters of 6 species are illustrated in Figure 1: 1 to 6 and summarized in Table 1.
The following observations were obtained: The average size of the pollen grains range from small (P/E= 25.2/15) in *N. deflersiana* Schweinf., to medium size (P/E= 36.3/30.3) in *S. aegyptiaca* L. (Table 1) which is quite different among the individual species, the genera, but not among the species of *Salvia*, and may confirm their position in different genera, this result agree with those reported by Harley et al. (1992), Jafari and Nikian (2008), Kahraman et al. (2009) and Özler et al. (2013). The shapes of pollen were described by using their polar and equatorial axis ratio which exhibit spheroidal, subspheroidal and prolate (Table 1). This result indicated that pollen shape can be used for the identification at genera and species levels which agree with that suggested by Perveen et al. (2003). The fine structure of the exine surface of pollen is quite uniform for almost all species under study as it is reticulate in general but it is reticulate-perforate in the pollen grains of *M. microphylla* Koch. (Figure 1: 1) and in *N. deflersiana* Schweinf. (Figure 1: 2) which agree with the results of the studies on the two genera *Mentha* L. and *Nepeta* L. (Celenk et al., 2008a, b). Also, it is reticulate-perforate in the pollen grains of *O. fruticosa* subsp. *schimperi* (Boiss.) Tackh. (Figure 1: 3) which is described in details for the first time in this work, and in general agreement with the exine ornamentations of the pollen of *O. aucheri* described by Perveen and Qaiser (2003). However, the exine sculpturing of the pollen is uniform as being bireticulate and perforate, in *S. deserti* (Figure 1: 4), *S. spinosa* (Figure 1: 5) and *S. aegyptiaca* (Figure 1: 6); This result

**Figure 1.** 1-6: SEM photographs of the pollen grains of Stachyoideae species, in polar view. 1: *Mentha microphylla*; 2: *Nepeta deflersiana*; 3: *Otoschegia fruticosa* subsp. Schimperi; 4: *Salvia aegyptiaca*; 5: *Salvia deserti*; 6 *Salvia spinosa*. 1, 4-6, hexacolpate. 2, 3: tricolpate.
agree with several investigations on pollen morphology of Salvia species (Hamzaoglu et al., 2005; Jafari and Nikian, 2008; Kahraman et al., 2009a, b, 2010a, b; Kahraman and Doghan, 2010; Dereboylu et al., 2010; Özler et al., 2013). The colpi of the pollen in the species investigated (Figure 1: 1-6) are two types: 1) Hexazonocolpate type in the pollen of M. microphylla Koch. (Figure 1: 1) and this is in accordance with those described in the genus Mentha L. (Celenk et al., 2008 a) and in the three Salvia species (Figure 1: 4, 5 and 6) which agree with those reported in the pollen grains of different Salvia species (Hamzaoglu et al., 2005; Jafari and Nikian, 2008; Kahraman et al., 2009a, 2010a, b; Dereboylu et al., 2010; Özler et al., 2013). 2) Trizonocolpate in the pollen of N. delersiana (Figure 1: 2) which is confirmed by Celenk et al. (2008 b) and O. fruticosa subsp. schimperi (Figure 1: 3) which agree with that reported by Perveen and Qaiser (2003). However, our results on limited desert species of subfamily Stachoideae showed useful morphological characters of pollen grains, e.g. shape, size colpi and fine structure of exine, which can be used in the identification of species and agree in general with previous investigations in Lamiaceae members in spite of the difference in their environment conditions.

Conflict of Interest

The authors did not declare any conflict of interest.

Table 1. Morphological characters of pollen grains of eight Stachyoideae species studied using LM and SEM.

<table>
<thead>
<tr>
<th>Species</th>
<th>Size mean value</th>
<th>P/E ratio</th>
<th>Shape*</th>
<th>Tectum surface</th>
<th>Number, type of colpi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mentha microphylla</td>
<td>26.8 ± 1</td>
<td>1.22</td>
<td>Sub-spheroidal</td>
<td>Reticulate perforate</td>
<td>6-Zonocolpate</td>
</tr>
<tr>
<td>Nepeta delersiana</td>
<td>25.2 ± 2</td>
<td>1.68</td>
<td>Prolate</td>
<td>Reticulate perforate</td>
<td>3-Zonocolpate</td>
</tr>
<tr>
<td>Otostegia fruticosa Subsp. schimperi</td>
<td>31.5 ± 2</td>
<td>1.10</td>
<td>Spheroidal</td>
<td>Reticulate Perforate Bireticulate Perforate</td>
<td>3-Zonocolpate</td>
</tr>
<tr>
<td>Salvia aegyptiaca</td>
<td>36.3 ± 2</td>
<td>1.19</td>
<td>Sub-spheroidal</td>
<td>Bireticulate perforate</td>
<td>6-Zonocolpate</td>
</tr>
<tr>
<td>Salvia deserti</td>
<td>36.5 ± 2</td>
<td>1.65</td>
<td>Prolate</td>
<td>Bireticulate perforate</td>
<td>6-Zonocolpate</td>
</tr>
<tr>
<td>Salvia spinosa</td>
<td>34.3 ± 2</td>
<td>1.24</td>
<td>Sub-spheroidal</td>
<td>Bireticulate perforate</td>
<td>6-Zonocolpate</td>
</tr>
</tbody>
</table>

*P/E ratio : Prolate = 1.33:2.00; Spheroidal = 0.88:1.14; sub-spheroidal = 0.75:1.33 (Punt et al., 2007). E, equatorial axis; P, polar axis. All measurements in µm.

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