Journal of Plant Sciences
2016; 4(3): 41-45
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20160403.12
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

Intra-Specific Pollen Size Variability in *Hyptis suaveolens* (L.) Poit. – an Ethnomedicinal Weed Taxon of Lamiaceae

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To cite this article:
Puja Rani Saha, Sangram Sinha, Rabindra Kumar Sinha. Intra-Specific Pollen Size Variability in *Hyptis suaveolens* (L.) Poit. – an Ethnomedicinal Weed Taxon of Lamiaceae. *Journal of Plant Sciences.* Vol. 4, No. 3, 2016, pp. 41-45. doi: 10.11648/j.jps.20160403.12

Received: April 6, 2016; **Accepted**: April 16, 2016; **Published**: April 28, 2016

Abstract: Variation in pollen stainability and morphometric sizes were recorded in the pollen grains of *Hyptis suaveolens* in a given sample population. Infrequent pollenkitt distribution in the outer wall of pollen was recorded. Distribution pattern of stained and unstained pollen revealed the characteristic variation in pollen sizes. The micromorphometric measurements of pollen grains were statistically analysed with one-way ANOVA and LSD among the mean values of pollen sizes. The results clearly revealed significant differences among the different categories of pollen sizes recorded in the present investigation. Present findings indicate the existence of pollen polymorphism with respect to size in *H. suaveolens* within the taxon.

Keywords: *Hyptis suaveolens*, Micromorphometry, Pollen Polymorphism, Pollenkitt, Lamiaceae

1. Introduction

Hyptis suaveolens (L.) Poit of the family Lamiaceae is a widely distributed obnoxious weed [22] and commonly called Pignut and locally known as Tukma in Tripura, India. Despite weediness, the species is reported to have ethnobotanical importance in traditional medicine and utility [3, 4, 14, 18, 27]. Morpho-taxonomical description of the plant habit of *H. suaveolens* was reported by different workers [7, 20] for identity of the species. *Hyptis suaveolens* is also described to have an astounding reproductive behaviour and reproduces both sexually as well as through vegetative means by perennating root stock. Genetic diversity is maintained through insect mediated cross pollination though self pollination is a part of reproduction in this species [2]. In spite of very special reproductive features, the species is reported to produce low natural seed output [1]. Pollen shedding during pollination is correlated with carinal explosion of the flower. Pollen grains are often shed at flower while anthers are still contained within the closed corolla. There is very limited information on morphometric studies of pollen grains of *H. suaveolens* in particular to pollen size variability and viability and as contribution to pollen polymorphism of the taxon. Intra and inter-specific polymorphism in pollen characters have been reported in certain members of Lamiaceae [10, 13]. The present study mainly thrust on pollen stainability, size and shape variability and distribution pattern in *Hyptis suaveolens*.

2. Materials and Methods

Hyptis suaveolens (L.) Poit. population of Suryamaninagar area, Tripura having geographical coordinates of 23º45'47.7" N and 91º16'02.5" E and an altitude of 18m was selected for the present study.Fresh flowering twigs were collected during the month of October, 2015 and herbarium specimens were prepared. The herbarium specimens were identified with the help of floristic literature [7, 11]. The herbarium specimen with accession number TU/B498 was submitted to the Herbarium of the Department of Botany, Tripura University for reference.

Mature bilobed anthers measuring an average length of 645.80 ± 52.48 µm were used as source of pollen grains in the present study. Micromorphometric measurements were carried out using ocular – stage micrometer method. Pollen size and stainability of *H. suaveolens* was studied following the standard aceto-carmine staining technique [23]. Not less than 1900 pollen grains were scanned for the stainability of the pollens. Measurements of polar axis (P), equatorial diameter (E) were taken randomly from 20 pollen grains of each different pollen size category under high power magnification (X 460) of the light microscope. The quotient P/E was worked out to determine the shape of the pollen based on Erdtman [8]. Pollen grain volume was also determined using the method described by Hrycan and Davis [12]. Pollen grains were grouped into different categories depending on size measurements of equatorial diameters and with reference to pollen categorisation reported by Walker and Doyle [24]. Distribution pattern of different categories of both stained and unstained pollen grains was also worked out. As many as 1000 pollens were scanned for analysis of distribution pattern of pollen grains.

Data were suitably analysed by Analysis of variance (ANOVA) and Fisher's Least significant difference (LSD) was used to compare the means of micromorphometric measurements.

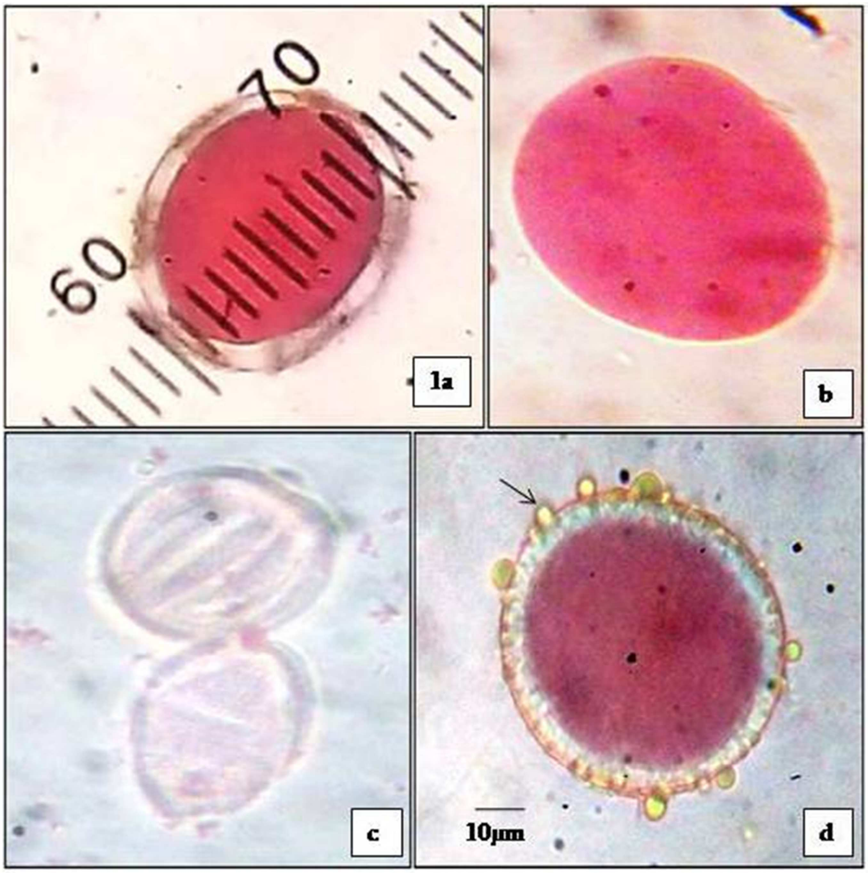
3. Results and Discussion

The present investigation mainly thrust on the pollen study with special reference to stainability, size and shape variability and distribution pattern. Microscopic observation of pollen grains clearly revealed 6 colpate in nature. Members of Lamiaceae are basically stenopalynous having the 3 or 6 colpate pollen and in particular 6 colpate in Hyptidinae [9, 21]. Out of 1900 pollens scanned, 72.32% stained and 27.68% unstained pollens were recorded. Difference in the stainability between thick and thin walled pollens was also observed (Figs. 1a - c). The outer thick walled pollens were found to have several distinct pollenkitt appearing as bright yellow liquid like sticky droplets (Fig. 1d). However not all pollens possess the pollenkitt. Significance of pollenkitt have been manifold and described for successful pollination in angiosperm [19]. Results on pollen stainability suggest that the considerable presence of unstained pollens may contribute towards the failure in fertilisation. Micromorphometric analysis of pollen grains recognised six different categories of pollens depending on stainability, wall thickness and size. Significant differences among the different categories of pollens clearly indicate the variability and existence of pollen polymorphism in the present population of *H. suaveolens*. Size differences of pollens within the same species have been reported in a few cases [15]. Polymorphism with respect to size and structural characteristics occurring in pollen grains of several species were also reported [13]. This variation in pollen size might be due to strong selection pressures related to pollen dispersal strategies [25, 16, 17]. Morphological variation in single pollen assemblage and a dozen abnormal or atypical types of pollen grains was reported in *Salvia leucantha* of Lamiaceae [10]. Mean pollen grain size in equatorial axis ranged from 29.63 ± 1.95 µm – 54.66 ± 9.49 µm and that of polar axis ranged from 26.12 ± 3.33 – 43.68 ± 4.38 µm recorded among the pollen categories (Table 1). This clearly suggests the existence of variability in pollen sizes. Earlier studies with members of Hyptidinae reported that pollens are oblate with an equatorial diameter of (27.5-) 40.0 (-60.0) µm and polar length of (24.8-) 33.9 (-49.7) µm and in particular the type "b" members of *Hyptis* [21]. Mean P/ E ratio in the present six different categories of pollen grains also revealed the characteristic suboblate type [8] in nature. However, data of individual observations for P/E quotient varied from 0.57 – 1.00 indicating occurrence of variable shapes of oblate, suboblate, oblate-spheroidal, spheroidal in this taxon. Present finding on different pollen shapes within a species is in accordance with previous records of *Salvia leucantha* of Lamiaceae [10]. The distribution of stained and unstained pollen types in 5 different size categories revealed that the most frequent category of stained pollen (56.96%) fall in the size range of 48.29µm - 57.07µm and designated as category III. In contrast, the highest percentage of unstained pollens (88.51%) was observed in category II with a size range 35.12µm – 43.90µm (Figs. 2a - b).These observations clearly indicate the pollen size variability among the stained and unstained pollens of *H. suaveolens.* Present observations also suggest that stained pollens of category III with highest frequency may constitute the major fertile pollens participating in pollination and consequently fertilisation. Chance encounter of unstained or non viable pollens in pollination leads to failure of fertilisation.

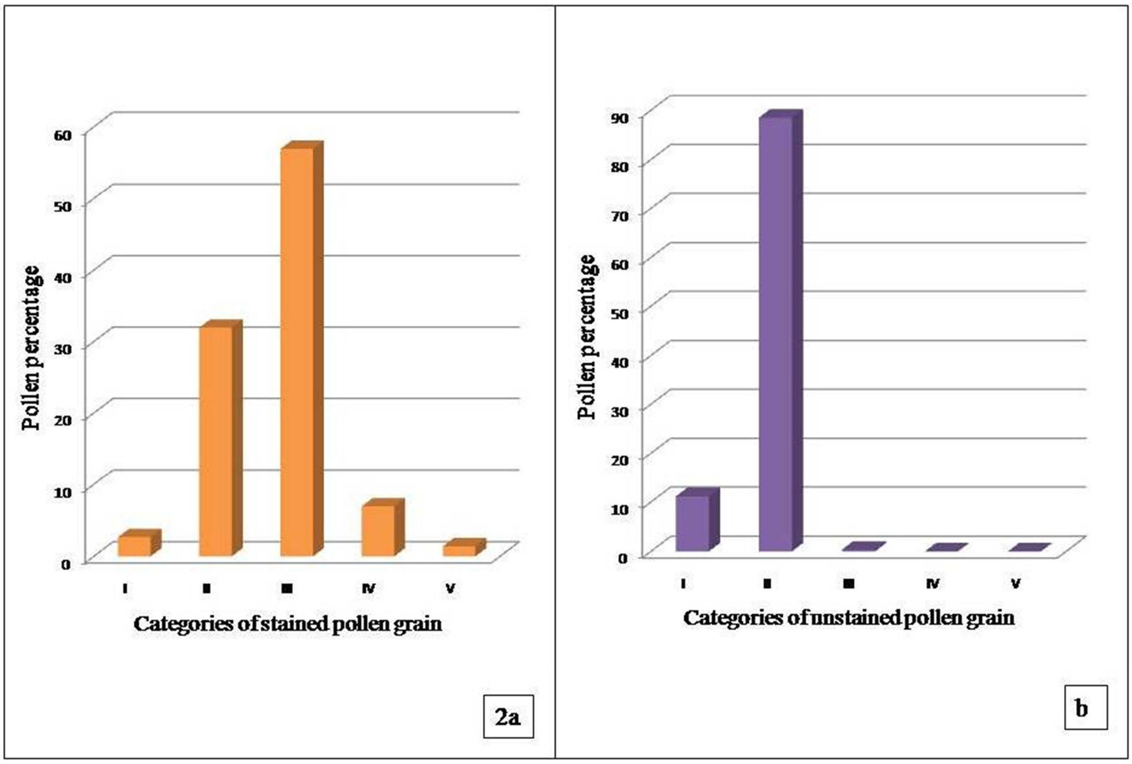
Table 1. *Micromorphometric measurements of Pollens of H. suaveolens.*

Pollen Size Category	**Equatorial length (E)**	**Polar length(P)**	**P/E ratio**	**Volume**
***Mean ± SD (µm)**	***Mean ± SD (µm)**	***Mean ± SD (µm)**	***Mean ± SD**	***Mean ± SD (µm³)**
Large stained with thick wall	49.83 ±2.58 (48.29-57.07)	43.68 ±4.38 (39.51-57.07)	0.87 ±0.10 (0.75-1.00)	$5.73\times10^4 \pm1.2\times10^4$
Large stained with thin wall	54.66 ±9.49 (39.51-70.24)	42.14 ±6.59 (30.73-57.07)	0.78 ±0.10 (0.57-1.00)	$6.99\times10^4\pm3.1\times10^4$
Medium stained	40.83 ±2.06 (39.51-43.90)	34.46 ±2.58 (30.73-39.51)	0.84 ± 0.06 (0.70-1.00)	$3.02\times10^4\pm4.2\times10^3$
Medium unstained	36.19 ±2.39 (30.73-39.51)	30.29 ±2.43 (26.34-35.12)	0.84 ±0.07 (0.75-1.00)	$2.10\times10^4\pm3.9\times10^3$
Small stained	32.05 ±3.52 (21.95-35.12)	26.78 ±2.81 (17.56-30.73)	0.84 ±0.08 (0.75-1.00)	$1.44\times10^4\pm3.2\times10^3$
Small unstained	29.63 ±1.95 (26.34-30.73)	26.12 ±3.33 (21.95-30.73)	0.88 ±0.10 (0.71-1.00)	$1.21\times10^4\pm2.5\times10^3$
LSD at 5%	2.37	2.08	0.04	7.2×10^3

*Mean of 20 replications, in parentheses () range of sizes, LSD = Least Significant Difference.



Figures 1. Pollens of Hyptis suaveolens. a) 6 colpate stained pollen with thick wall, b) Large stained thin walled pollen, c) Unstained pollens in equatorial view, d) Pollen with distinct yellow Pollenkitt.



I: (17.56 – 30.73) µm, II: (35.12 – 43.90) µm, III: (48.29 – 57.07) µm, IV: (61.46 – 70.24) µm, V: (74.63 – 92.19) µm.

Figures 2. Distribution pattern of stained and unstained pollens of H. suaveolens with respect to their size range. a) Categories of stained pollens, b) Categories of unstained pollens.

Diversification of pollination systems and diverse ranges of pollen types with respect to stainability, wall nature, and size may render as significant factors in success of pollination and fertilisation. Pollen is one of the major genetic factors contributing in sexual reproduction resulting in fruit and seed production. It may be predicted that the variability in pollen types might account for the variations recorded in the type of seed output and seed dimorphism [26] that was reported in this species. Reduced fertility of smaller pollen compared to larger one in *Erythronium grandiflorum* was reported due to less stylar attrition [5]. Pollen polymorphism in *Thymus capitatus* has been related to environmental parameters and mainly with water availability [13]. In the present study, it may also be predicted and suggested that one of the possible cause of pollen polymorphism recorded in *H. suaveolens* might be due to environmental constraints prevailing in the population site of the present taxon. Moreover, variable pollen size classes with infrequent pollenkitt distribution and variable wall thickness may also be attributed due to its strategic high adaptability in diverse habitats [20].

4. Conclusion

Study on acetocarmine stainability revealed 72.32% stained and 27.68% unstained pollen grains in a given sample population of *Hyptis suaveolens*. The pollen shape and sizes were determined under Light microscope. Considerable variation of pollen grains was recorded and described into six different categories depending on size, stainability and wall thickness. Most frequent category of stained pollen (56.96%) was found to have a size range of 48.29µm – 57.07µm with infrequent pollenkitt distribution in the outer wall. Distinct pollen polymorphism in size of *H. suaveolens* was recorded and found significant among the different pollen categories.

Acknowledgements

The first author is highly thankful to University Grants Commission, Government of India for providing financial support to carry out the present piece of research work under the UGC - RET fellowship programme of Tripura University.

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