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RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF CALCIUM OXALATE CRYSTALS OF THREE EDIBLE TAXA IN SOUTH WEST BENGAL, INDIA

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ABSTRACT

Calcification and the formation of crystals is a common phenomenon in many plant groups under normal conditions. In Angiosperms, calcium oxalate is predominantly deposited as calcium salt and the most commonly encountered shapes include the raphides, druses, styloids, prismatic and crystal sands. They are varies from species to species or with in species, mainly occurs sporadically in all organs. Raphides (long, slim, pointed crystals) were most common, but druses (crystal aggregates) were also found in most of the plant organ, which also give mechanical support, mineral balance, waste sequestration, and protection against herbivores have all been proposed as crystal functions. Raphides have long been associated with the acridity of the aroids, meaning that when eaten raw they cause swelling of the lips, mouth and throat. Detoxification via cooking, pounding or leaching neutralizes the chemical, hence making the aroids edible, but does not destroy or degrade the raphides The edibility is depend upon the distribution and frequency of calcium oxalate crystals (COCs).

INTRODUCTION

Raphides most commonly occur in bundles of tens to hundreds of crystals in specialized cells called idioblasts (Fig. 4, 5, 8, 11 and 14) which differ markedly from neighboring cells **(**Foster, 1956**)**. Among the most common idioblasts are those that synthesize crystals of calcium oxalate **(**Franceschi and Horner, 1980; Horner and Wagner, 1995; Franceschi and Nakata, 2005). Many crystal forms are known **(**Franceschi and Horner, 1980**)** including druses, which are spherical crystal aggregates; raphides, which are long pointed needles found in bundles within cells.

Although less common, they are also known to form in extracellular bundles **(**Barabé, Lacroix, Chouteau and Gibernau, 2004**)** and there has been one report of raphides forming within starch granules (Okoli and Green, 1987**).** Idioblast cells are structurally modified to accommodate crystals and are therefore markedly different in form, structure, and contents from other cells in the same tissue **(**Sunell and Healey, 1981**).** But in some cases idioblasts also found in arranged condition in a vacuole (Saadi and Mondal, 2012). A variety of idioblast cell forms have been identified **(**Keating, 2004**)** which can be grouped broadly into those having defensive or non-defensive functions **(**Sunell and Healey, 1985**)**. The latter form crystals in relatively loose arrangement compared to defensive cells, which are usually smaller in size and suspended in the intercellular airspaces from where they are easily dislodged **(**Sunell and Healey, 1985**)**. When a defensive idioblast is disrupted, the tightly packed raphides are ejected through thin-walled papillae at its ends, a feature likened to an 'automatic microscopic blowgun' (Fig.5) (Middendorf, 1983; Saadi and Mondal, 2012**)**. This mechanism probably developed as a defence against herbivore McNair, (1932) listed 215 families in which they had been reported. Crystals are particularly diverse in the Araceae **(**Prychid and Rudall, 1999**)** and their distribution in this family has been reviewed **(**Keating, 2003**;** Keating, 2004; Saadi and Mondal, 2012**)**. Despite the ubiquity and variety of crystals in plants, the purpose of these structures is poorly understood. It is often assumed that crystals might deter herbivory. However, whether crystals are truly effective as deterrents has not been clearly demonstrated. Furthermore, it is not known whether all crystal types can deter herbivores or whether crystals have other important roles. The formation of calcium oxalate crystals (COCs) (Fig. 2, 6, and 10) is a basic and important process of many plant species. I here describe the variety of crystal found within vegetative organs like-leaf, petiole and storage organ or corm of *Colocasia esculenta* (Linn.) Schott, *Amorphophallus paeoniifolius* (Dennst.) Nicolson and *Alocasia macrorrhizos* (L) G.Don and these crystals is important indicator of the family Araceae, which is found in different plant parts in different forms, e.g. Raphides and Druses. The edibility of these parts depends upon the frequency of these cryatals. High frequency of the crystals increase, the toxicity and when eaten raw they cause swelling of the lips, mouth and throat. Detoxification via cooking, pounding or leaching neutralizes the chemical, hence making the aroids edible.

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MATERIALS AND METHODS

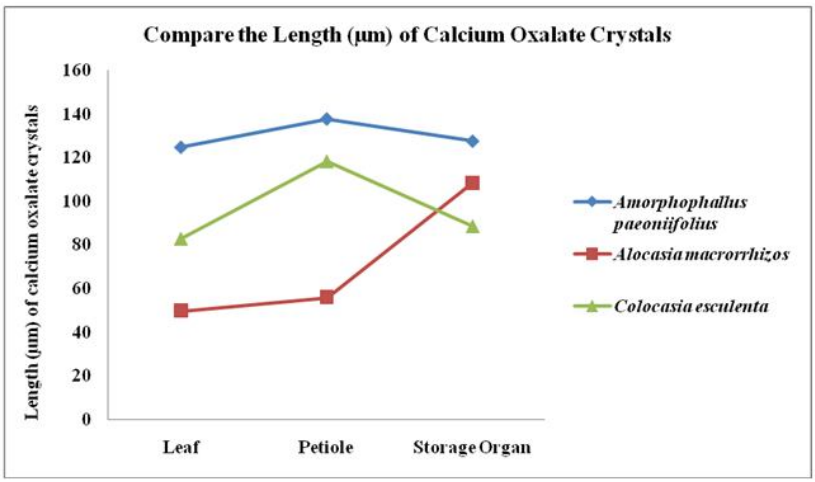
The plant specimens belonging the family Araceae. The species identification of the selected materials was determined according to standard literature. It was done in the month February to May.

Tissue-preparation and microscopy

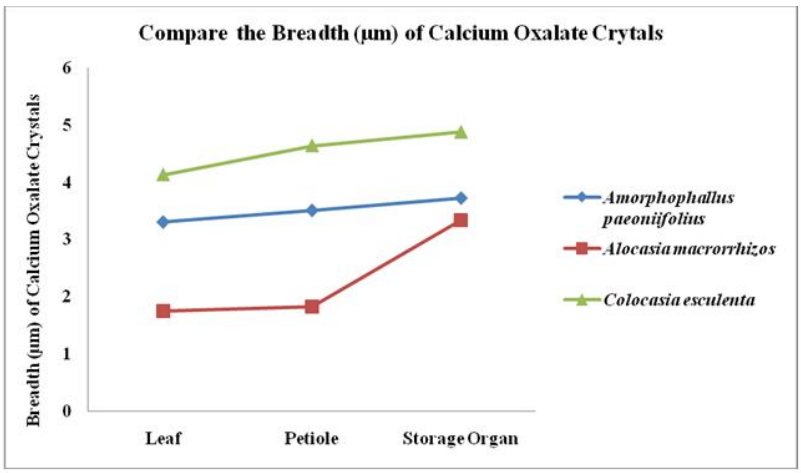
Organs were hand-sectioned with a single-edged razor blade. Transverse sections were prepared of leaf, petiole and storage organ or corm. Petioles and leaf midveins were cross-sectioned. Leaves and petiole wings were cut into pieces about 1 cm^2 or smaller. All samples were cleared using a modification of the NaOH tissue clearing method of Ruzin (1999) as follows. They were placed in 5% NaOH and shaken on a rotary platform shaker at 30 rpm for 2 to 4 days. Sufficient liquid was used to allow free movement of the tissue

Table 1. Length and Breath of Calcium Oxalate Crystals (Raphides) of Selected Species

SL. NO	SCIENTIFIC NAME OF THE PLANT	LEAF				PETIOLE				STORAGE ORGAN OR CORM			
		L (µm).		B(µm)		L (µm).		B(µm)		L (µm).		B(µm)	
1.	*Colocasia esculenta* (Linn.) Schott.	83.868	82.95	3.975	4.13	121.952	118.13	3.982	4.64	110.916	88.67	4.600	4.88
		84.960		4.185		112.687		5.489		108.001		4.798	
		80.025		4.236		119.752		4.456		47.097		5.236	
2.	*Alocasia macrorrhizos* (L) G.Don	52.134	49.73	1.775	1.75	56.247	56.07	1.978	1.83	108.245	108.37	3.252	3.34
		43.985		1.519		56.060		1.896		108.364		3.518	
		53.083		1.952		55.915		1.626		108.528		3.252	
3.	*Amorphophallus paeoniifolius* (Dennst.) Nicolson	123.831	124.63	3.140	3.31	154.814	137.70	3.851	3.51	126.437	127.45	3.648	3.72
		127.991		3.203		151.225		3.408		128.721		3.951	
		122.063		3.598		107.036		3.269		127.198		3.554	



Graph 1. Compare the Length (µm) of Calcium Oxalate Crystals



Graph 2. Compare the Breadth (µm) of Calcium Oxalate Crytals

samples, generally about 30 ml. Samples were then bleached in full strength household chlorine bleach (3–6% sodium hypochlorite), usually for 5–10 min, but no more than 30 min, and then washed three times in water for at least 10 min each time. Samples were maintained on the shaker at 30 rpm during bleaching and washing. Cleared samples were then dehydrated by passing them through 25%, 50%, 75%, and 100% reagent grade ethanol, and rinsed again in 100% ethanol. Each solution was applied for at least 10 min with shaking at 30 rpm. They were then placed in 1:1 reagent grade ethanol: xylene (xylol) under a fume hood for 10 min with occasional swirling, and then in two changes of xylene for 10 min each, with swirling, under the fume hood. Finally, the samples were transferred to the microscope slide and covered with a glass cover slip. Slides were examined with bright field and polarization microscopy. The latter, viewing the slides through polarizing sunglasses. The filters on the light source were rotated to achieve extinction of background illumination when viewed through the sunglasses. Digital micrographs were taken with a Nikon (NIKON ECLIPSE (LV100 POL)) digital camera mounted on a Nikon Eclipse 50i microscope (Nikon, Tokyo, Japan). Some low magnification digital micrographs were taken with an light microscope (10X x 40X) (Olimpus), Phase Contrast Microscope (Leica DM- 1000)

RESULT

Idioblasts containing raphides (cocs)

Raphides released by disrupting leaf tissue in a mortar and pestle, or in a blender, after then we measure the length and breadth (Phase Contrast Microscope (Leica DM-1000)) of raphide (Table 1, Graph 1 and 2) in leaf, petiole and storage organ or corm in which all the cases variation of length and breadth occur. The following are-

Leaf, length of raphides is longer in *Amorphophallus paeoniifolius* (124.63µm), shorter in *Alocasia macrorrhizos* (49.73µm). Breadth of raphides is greater in *Colocasia esculenta* (4.13µm), smaller in *Alocasia macrorrhizos* (1.75 µm).

Petiole, length of raphides is longer in *Amorphophallus paeoniifolius* (137.70µm), shorter in *Alocasia macrorrhizos* (56.07µm). Breadth of raphides is greater in *Colocasia esculenta* (4.64µm), smaller in *Alocasia macrorrhizos* (1.83 µm).

Storage organ or corm, length of raphides is longer in *Amorphophallus paeoniifolius* (127.45µm), shorter in *Colocasia esculenta* (88.67µm). Breadth of raphides is greater in *Colocasia esculenta* (4.88µm), smaller in *Alocasia macrorrhizos* (3.34 µm).

IDIOBLAST

We found two types of crystal idioblasts, one is raphide idioblasts and other is druse idioblasts.

Raphide idioblast

Spindle-shaped idioblasts with terminal nipples, capable of ejecting their crystals, **(**Fig. 5) could be easily recognized in leaves, and other tissues because they generally expelled a portion of their crystals during tissue clearing and were arranged parallel in cylindrical bundles. In case of petiole the giant idioblast are arragened in a ring within the vacuole (Fig. 11) and sometime idioblast found oppositely in the cell layer in between two vacuoles. (Fig. 4)

Druse idioblasts

Each druse is an aggregate of crystals which have variable morphology (Fig. 1, 9 and 12). Druses may have a similar defensive function to that of raphides, as they also have sharp points resulting in considerable irritation if eaten. Druses generally resemble with adjacent cells in size and shape**.**

DISTRIBUTION OF CRYSTALS WITHIN PARTICULAR TISSUES

Leaves

Leaf blades contained a variety of crystal idioblasts (Fig.13), Manipulating the focus at high power showed that most crystals, of all types, were distributed primarily within the upper mesophyll, with only occasional crystals coming into focus lower in the leaf. Druses were the most common crystal type in leaves (Fig. 9 and 12), and occasionally very small druses were also seen. Raphide- giant bundles, and those containing bundles of small raphides. The small bundles varied from tightly to loosely bundled. Different parts of the leaf blade seemed to contain the same kind of crystal idioblasts, in similar densities, except that leaf margins were marked by excess druses and other raphide bundles. A leaf margin is increased density of druses along the immediate edge can be seen.

Petiole

In case of petiole the raphide idioblasts are arranged in a ring they are mostly non-defensive (Fig. 3, 8 and 11). Beneath the stem epidermis was a layer of parenchyma which contain druses and giant bundles (Fig. 8).The cortical cells outside this crystal-containing layer, and the longitudinally lengthened collenchyma cells inward from this layer contained few or no crystal idioblasts. The internal pith of stems contained many druses and small raphide bundles.

Storage organ or corm

The epidermal surface of the storage organ have highest frequency of raphide and druse idioblasts than the inner portion of storage organ. Most of idioblasts are non-defensive. The Non-defensive idioblasts found in scattered condition in the surrounding tissue (Fig. 14).

THE VARIATION IN THE NUMBER OF DRUSE AND RAPHIDE IDIOBLASTS IN PER MICROSCOPIC FIELD

The observation revealed that the leaf, petiole and storage organ or corm of different species like- *Colocasia esculenta* (Linn.) Schott, *Amorphophallus paeoniifolius* (Dennst.) Nicolson, and *Alocasia macrorrhizos* (L) G.Don have different frequency of calcium oxalate crystals like- raphides and druses taken by NIKON ECLIPSE (LV100 POL) in 10X.(Table II, Graph-III and IV) The variation of raphide idioblasts (Fig 13) was found to be highest in the leaves (8.66) of *Amorphophallus paeoniifolius*; Petiole (10.66) of *Colocasia esculenta* and Storage organ or corm (15.33) of *Amorphophallus paeoniifolius*, while minimum variation was found in the leaves (7.33) of *Alocasia macrorrhizos*; Petiole (3.66) of *Amorphophallus paeoniifolius* and Storage organ or corm (5.33) of *Alocasia macrorrhizos*. The variation of druses idioblasts (Fig 7) was found to be highest in the leaves (56.66) and petiole (25) of *Colocasia esculenta* and Storage organ or corm (328.33) of *Alocasia macrorrhizos* while minimum variation was found in the leaves (7) and storage organ or corm (4) of of *Amorphophallus paeoniifolius* and petiole (7.66) of *Alocasia macrorrhizos*.

DISCUSSION

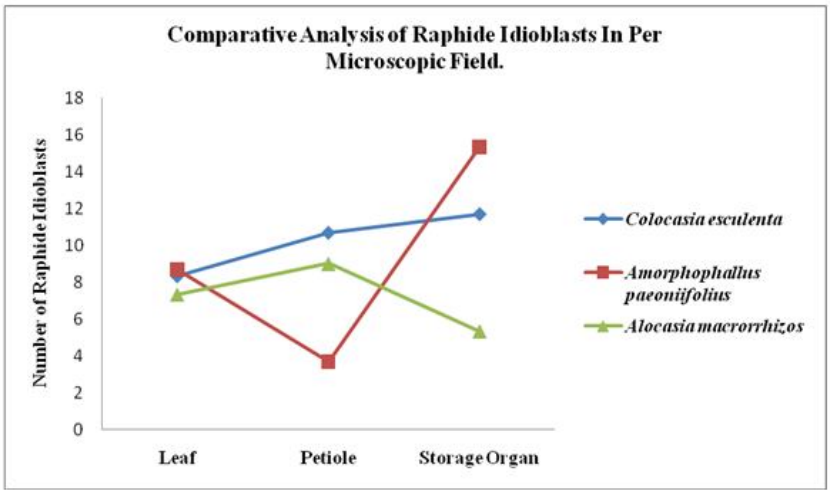
Two basic crystal forms were found: druses (Fig. 1 and 12) and raphides (Fig. 10), which found essentially throughout the plant, Raphides showed diversity in their arrangement within the idioblast cell, from neatly bundled to somewhat disorganized. Raphides occur in at least two thicknesses and a diversity of lengths. Druses also come in different sizes, in some case larger, and occasionally, very small druses in different plant organs. Some crystal types could represent developmental variation. Small or less organized raphide bundles, The variety of precise crystal morphologies in 3 selected plants generally, which are not seen in commercially crystallized calcium oxalate **(**Webb, 1999**)** suggests that plants impose the morphology upon the developing crystal **(**Arnott and Pautard, 1970**).** Idioblasts produce crystals by co-crystallizing oxalate and calcium ions within membrane-bound crystal chambers **(**Webb, 1999**)** Particular proteins and/or carbohydrates within the crystal chamber might further control crystal development **(**Webb, 1999**).** The diversity of crystal morphologies here described for that the species must have different crystal developmental programs in different organs and even in different cells within the same organ. Along with great diversity, crystals in selected plants show tissue specificity; each tissue has a particular complement of crystals. For example, while druses appear to be nearly ubiquitous throughout the, different portions of the same organ may have different crystal types. For example, leaf margins have a greater density of druses than the lamina, as well as overlapping raphide.

Roles of the calcium oxalate crystals (COCs)

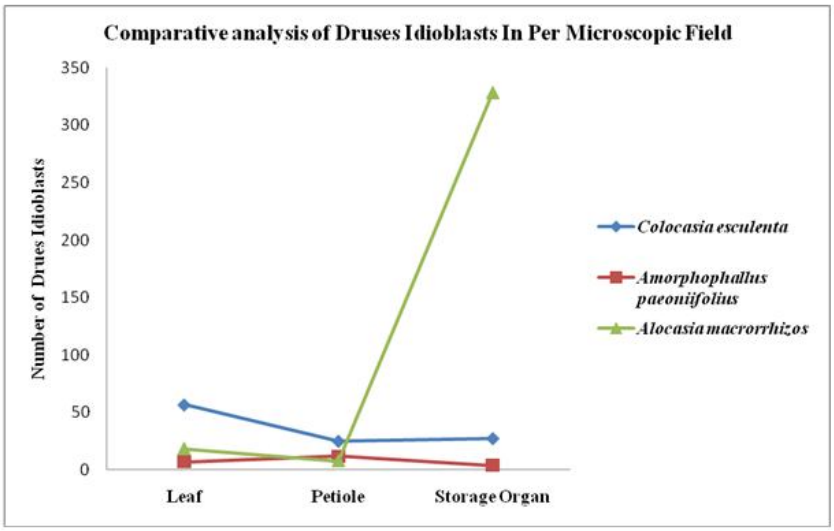
A number of roles have been suggested for crystal-containing idioblasts **(**Arnott and Pautard, 1970; Franceschi and Nakata, 2005**).** The crystals could provide long-term storage of calcium because the crystals apparently can be mobilized and degraded as needed **(**Franceschi and Horner, 1980**;** Franceschi, 1989 and Webb, 1999**).** The crystals might also serve as a calcium sink, immobilizing excess calcium because plants regularly absorb more calcium than needed **(**Webb, 1999**).** It has been suggested that the crystals could function as deterrents to herbivores **(**Franceschi and Horner, 1980**).** In this role, the crystals certainly capture the imagination. Chewing druses would be like chewing sand, and the needle-like raphides would be ideal for penetrating herbivore mouthparts. The ability of biforines to forcibly expel their raphides could function in vivo to drive crystals into the tissues of anything chewing on them. It has been suggested that proteolytic enzymes **(**Walter and Khanna, 1972**)**, other toxic

Table 2. Frequency of raphide & druse idioblast in different plant parts in the selected members of araceae in per microscopic field:

S.No.	Scientific Name of the Plant	Plant parts	Raphide Idioblast Per Microscopic Field (10X)		Druses Idioblast (10X)	
1	*Colocasia esculenta* (Linn.) Schott	Leaf	9		55	
			11		46	56.66
			5	8.33	69	
		Petiole	9		22	
			11		28	25
			12	10.66	25	
		Storage organ	11		23	
			14	11.66	31	
			10		28	27.33
2	*Amorphophallus paeoniifolius* (Dennst.) Nicolson	Leaf	9		7	
			7	8.66	6	7
			10		8	
		Petiole	3		14	
			5		10	
			3	3.66	11	11.66
		Storage organ	21		2	
			13	15.33	5	4
			12		5	
3	*Alocasia macrorrhizos* (L) G.Don	Leaf	9		22	
			8	7.33	18	18.33
			5		15	
		Petiole	12		6	
			8	9	9	7.66
			7		8	
		Storage organ	8		397	
			5	5.33	340	328.33
			3		248	



Graph 3. Comparative analysis of Raphide Idioblasts In Per Microscopic Field



Graph 4. Comparative analysis of Druses Idioblasts In Per Microcopic Field

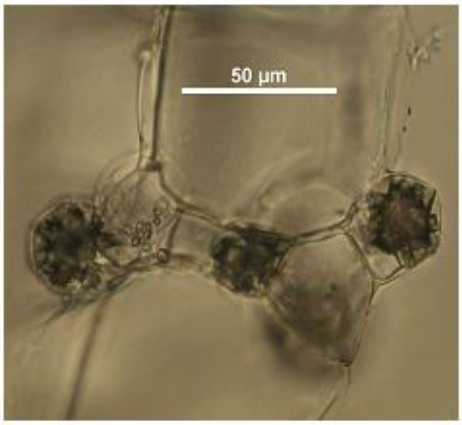
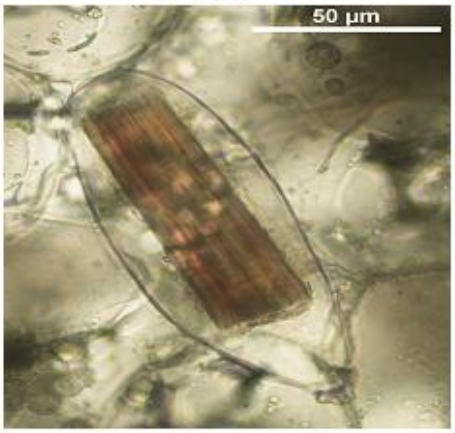


Fig-1

Fig-2



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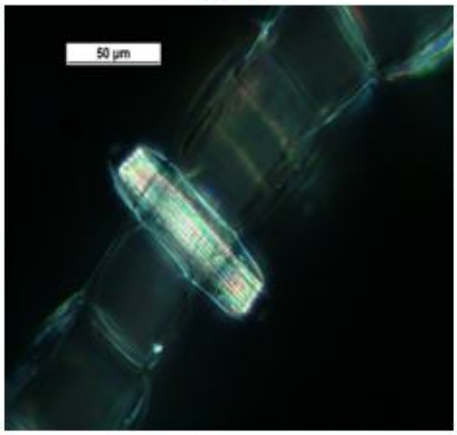


Fig-4

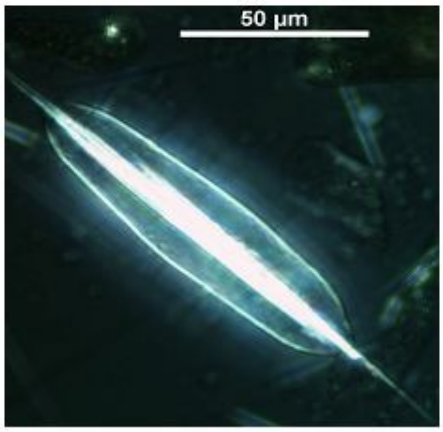
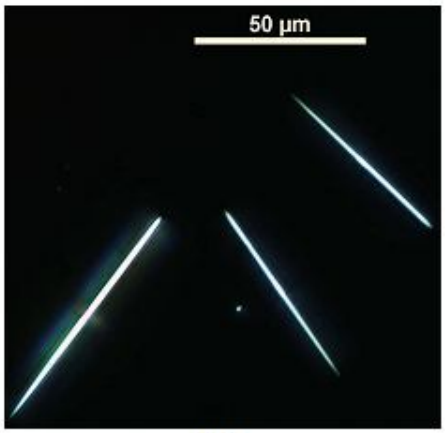


Fig -5



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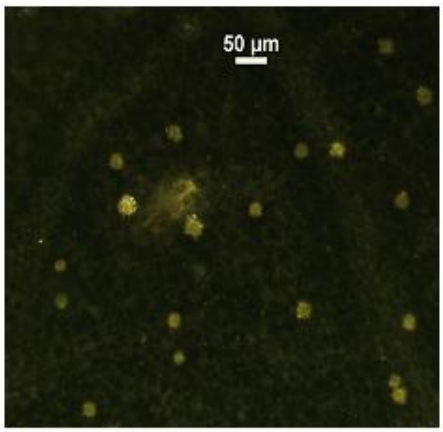

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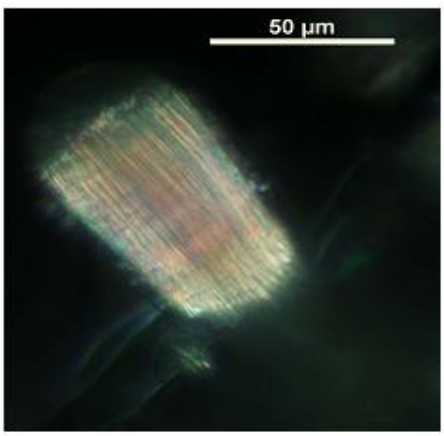

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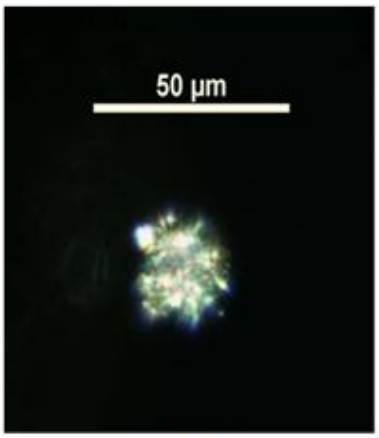

Fig-9

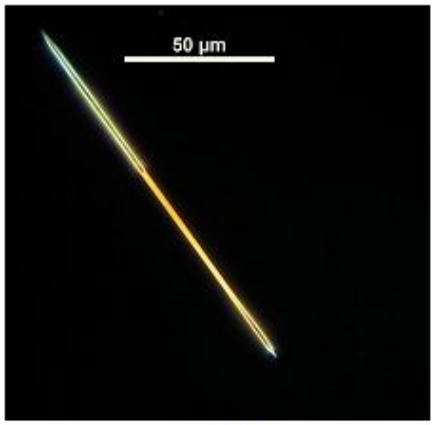

Fig-10

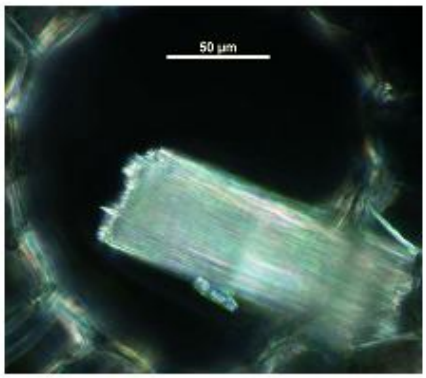

Fig-11

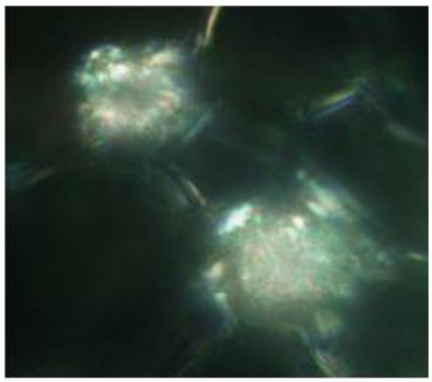

Fig-12

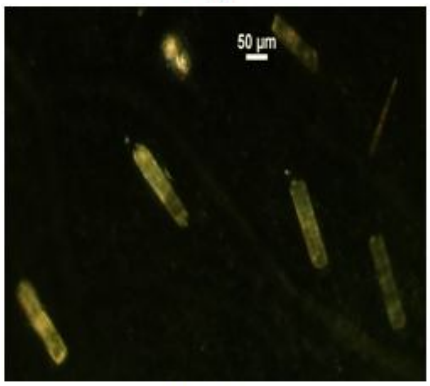

Fig-13

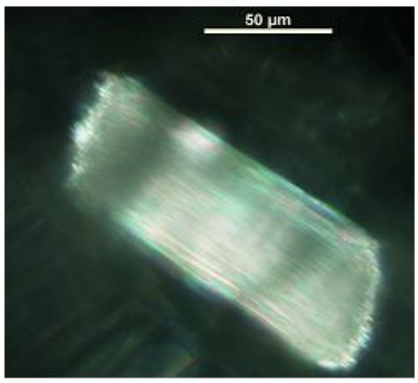

Fig-14

Fig- 1. Druses of petiole in *Colocasia esculenta* 2. Raphides of petiole in *Colocasia esculenta* 3. Non-defensive idioblast of storage organ or corm in *Colocasia esculenta* 4. Defensive idioblast of petiole in *Colocasia esculenta* 5. Defensive idioblast of in *Colocasia esculenta* 6. Raphides of leaf in *Alocasia macrorrhizos* 7. Frequency of druses in the leaf of *Alocasia macrorrhizos* 8. Non-defensive idioblast of *Alocasia macrorrhizos* 9. Druse of leaf in *Alocasia macrorrhizos* 10. Raphides of leaf in *Amorphophallus paeoniifolius* 11. Non-defensive idioblast of petiole in *Amorphophallus paeoniifolius* 12. Druse of petiole in *Amorphophallus paeoniifolius* 13. Frequency of leaf in idioblast of *Amorphophallus paeoniifolius* 14. Non-defensive dioblast of storage organ or corm in *Amorphophallus paeoniifolius*.

proteins (Fochtman, Manno, Winek a nd Cooper, 1969), glucosides **(**Saha and Hussain, 1983**)** or other toxins may be incorporated into the organic matrix surrounding the crystal. Crystal-bearing aroids, is reported to be poisonous to humans, causing oral irritation and inflammation, **(**Arditti and Rodriguez, 1982; Gardner, 1994**)**. Indeed, taro (*Colocasia esculenta*) and konjac (*Amorphophallus konjac*), among other aroids, are grown for human consumption in the tropics, although they must be dried or cooked before they can be eaten. Drying or cooking prevents active ejection of the crystals and could conceivably also destroy any poisons associated with the crystals, especially proteins. The selected plant organs contain a diversity of calcium oxalate crystals differing in morphology, size, and tissue localization. It seems likely that they might play a number of different roles in the plant. The raphides and druses might deter herbivores. The edibility is depend upon the distribution and frequency of calcium oxalate crystals (COCs) and high frequency of calcium oxalate crystals containing edible plant parts is not edible because high frequency crystals increase the toxicity. So, by the different process if we decrease the toxicity by cooking or cooling then the storage organ becomes edible.

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REFERENCES

Arditti J, and Rodriguez E (1982). *Dieffenbachia:* Uses, abuses and toxic constituents: A review. *Journal of Ethnopharmacology* 5: 293–302.

Arnott H J, and Pautard FGE (1970). Calcification in plants. *In* H. Schraer (ed.), Biological calcification: Cellular and molecular aspects, 375–446. Appleton-Century-Crofts, New York, New York, USA.

Barabé D, Lacroix C, Chouteau M, and Gibernau M (2004). On the presence of extracellular calcium oxalate crystals on the inflorescences of Araceae. *Botanical Journal of the Linnean Society* 146: 181–190.

Fochtman FW, Manno JE, Winek CL and Cooper JA (1969. Toxicity of the genus *Dieffenbachia. Toxicology and Applied Pharmacology* 15: 38–45.

Foster AS (1956). Plant idioblasts: Remarkable examples of cell specialization. *Protoplasma* 46: 184–193.

Franceschi VR and Horner HT Jr (1980). Calcium oxalate crystals in plants. *Botanical Review* 46: 361–427.

Franceschi VR and Nakata PA (2005). Calcium oxalate in plants: Formation and function. *Annual Review of Plant Biology* 56: 41–71.

Franceschi VR (1989). Calcium oxalate formation is a rapid and reversible process in *Lemna minor* L. *Protoplasma* 148: 130–137.

Gardner DG (1994). Injury to the oral mucous membranes caused by the common houseplant, dieffenbachia: A review. *Oral Surgery, Oral Medicine, and Oral Pathology* 78: 631–633.

Horner HT Jr. and Wagner BL (1995). Calcium oxalate formation in higher plants. In S. R. Khan (ed.),*Calcium Oxalate in Biological Systems*, pp 53–72. Boca Raton: CRC Press.

Keating RC (2003). Anatomy of the monocotyledons. IX. Acoraceae and Araceae. Oxford University Press, Oxford, UK.

Keating RC (2004). Systematic occurrence of raphide crystals in Araceae. *Annals of the Missouri Botanical Garden* 91:495–504.

McNair JB (1932). The interrelation between substances in plants: Essential oils and resins, cyanogen and oxalate. *American Journal of Botany* 19: 255–272.

Middendorf EA (1983). The remarkable shooting idioblasts. *Aroideana* 6:9–11.

Okoli BE and Green BO (1987). Histochemical localization of calcium oxalate crystals in starch grains of yams (*Dioscorea*). Annals of Botany 60:491–394.

Prychid CJ and Rudall PA (1999). Calcium oxalate crystals in monocotyledons: A review of their structure and systematics. *Annals of Botany* 84:725–739.

Ruzin SE (1999). Plant microtechnique and microscopy, Oxford University Press, New York, New York, USA.

Saadi SMAI and Mondal AK (2012). Distribution of calcium oxalate crystal containing idioblasts in the leaves of *Syngonium podophyllum Schott*. *Int. J. LifeSc. Bt and Pharm. Res.* 1: 227-235.

Saadi SMAI and Mondal AK (2012). Distribution of calcium oxalate crystal containing idioblasts in the leaves of *Aglaonema commutatum* Schott. *International Journal of Science and Nature*. 3: 286-292.

Saadi SMAI and Mondal AK (2012). Studies on the calcium oxalate crystals (Raphides) and idioblast of some selected members of Araceae in Eastern India. *African Journal of Plant Science* 6: 256-269.

Saha BP and Hussain M (1983). A study of the irritating principle of aroids. *Indian Journal of Agricultural Sciences* 53: 833–836.

Sunell LA and Healey PL (1981). Scanning electron microscopy and energy dispersive X-ray analysis of raphide crystal idioblast in taro. *Scanning Electron Microscopy* 3: 235±244.

Sunell LA and Healey PL (1985). Distribution of calcium oxalate crystal idioblasts in leaves of taro (*Colocasia esculenta*). *American Journal of Botany* 72:1854–1860.

Walter WG and Khanna PN (1972). Chemistry of the aroids. I. *Dieffenbachia seguine, amoena* and *picta*. *Economic Botany* 26: 364–372.

Webb MA (1999). Cell-mediated crystallization of calcium oxalate in plants. *Plant Cell* 11: 751–761.

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