J. Asiat. Soc. Bangladesh, Sci. 41(1): 59-66, June 2015

CRYSTAL FORMATION IN CULTURES OF FUNGI : NEW RECORDS FROM BANGLADESH

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Abstract

In the present study crystal formations were detected in the culture of *Aspergillus niger*, *A. fluvus*, *Aspergillus* sp., *Bipolaris sorokiniana, Cladosporium* spp., *Colletotrichum gloeosporioides, Colletotrichum orbiculare,Curvularia* spp., *Fusarium* sp., *Paecilomyces* spp., *Penicillium* sp., *Pestalotia* sp., *Pestalotiopsis guepinii*, *Phaeoisariopsis personata*, *Trichoderma viride* and seven sterile culture of fungi. Eighteen types of crystallographic structures were detected from different fungal isolates. This is the first report of formation of crystal in fungal cultures from Bangladesh.

Key words: Crystal, Culture, Fungi, Bangladesh

Introduction

Occurrence of crystals was considered as a rarity in the world of fungi because their existence was, at that time (1880), known only in the Phycomycetes studied first by Klein (1872) and later by Van Tieghem (1878). Zopf (1890) referred to the work of Van Tieghem (1878) who found crystals in cultures of quite a number of Fungi Imperfecti. Production of crystals by fungi has also been reported by de Bary (1887).

Despite the identical chemical composition of the crystals, their crystallographic form shows the greatest variability (Borzani 1959). In the language of crystallographer crystal may be acicular (very slender forms), capillary, radiated, satellite, columnar, rosette and many other form. Previously, crystals had been recorded in Dermatophytes namely, *Keratinomyces ajelloi* (4 strains), *Microsporon*, n. sp. *Microsporon audouinii* (the genus *Microsporon* is synonym of *Mycosporum*), *Epidermophyton floccosum, Mixotrichum conjugatum* and *Rozites gonglyophora.* In Hyphomycetes, *Aspergillus fumigatus* and *A. fluvus* produced crystal (Benedek 1961, Borzani 1959, 1960 and Borzani *et al.* 1960).

Lot of researches had been carried out on crystals in fungal culture and its chemical composition (Graustain *et al*. 1977, Punja and Jenkins 1984, Smith *et al*. 1986 and Arnott 1995). Recently different types of crystals were observed in sporulating fungal culture. Since there is no report on this regards in Bangladesh, the present investigation

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was undertaken to record the different types of crystal forms as they occur in fungal cultures.

Materials and Methods

The fungi were isolated from healthy and diseased plant parts following "Tissue planting" and "Blotter" methods (Tuite 1969). Some air borne fungi isolated on Potato Dextrose Agar (PDA) plate were found to produce crystals in cultures at 25-28 ^{0} C and pH 6, within 7 days of incubation. Sporulating fungal cultures showed crystal in slide. Fungal structure like mycelia, spore bearing structures and spores were scrapped off from the surface of the sporulating colony with a scalpel or blade or picked up with a needle and was mounted in lacto phenol over a clean slide for microscopic observation. In case of hyaline structures, a little amount of aniline blue (cotton blue) was added to the mounted fluid. Crystals may be found as early as the seventh day and older culture contain more crystals.

Crystals ware observed with fungal structures under Binocular microscope with normal light. Under microscopic view different types of crystals were observed in different fungal species. The isolated fungi were identified based on morphological characteristics observed under a compound microscope following standard keys (Barnett and Hunter 2000, Booth 1971, Ellis 1971, 1976, Ellis and Ellis 1997 and Sutton 1980).

Results and Discussion

The diverse crystallographic structures found in the cultures of Deuteromycetous fungi were isolated during July 2009 to May 2014. In total 20 species of fungi belonging 11 genera of Deuteromycetes and seven sterile fungi were found to produce crystals in cultures (Table 1). The different strains of the same species of the fungi may produce the different crystallographic forms (Plates 1 to 3). On the other hand, the same crystallographic forms may occur in cultures of different, unrelated species of fungi (Table 1). The crystal forms may be entirely different in the same species (Table 1 and Plate 4). English and Jensen (1958) mentioned that crystals are the flowers of mineral kingdom.

In the present study crystal formations were detected in the culture of *Aspergillus flavus* Link., *A. niger* Van Tiegh., *Aspergillus* sp., *Bipolaris sorokiniana* (Sacc.) Shoem,, *Cladosporium* spp. *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., *Colletotrichum orbiculare*, *Curvularia* spp., *Fusarium* spp., *Penicillium* sp., *Pestalotia* sp., *Pestalotiopsis guepinii* (Desm.) Stayaert, *Phaeoisariopsis personata* (Berk and M.A. Curtis) Arx., *Paecilomyces* sp., *Trichoderma viride* Pers. and seven sterile fungi.

Table 1. Different crystallographic types associated with different fungi isolated from different hosts/ habitats.

Name of Fungi	Crystal Type	Host/Habitat
Aspergillus niger	Radiated	On air
Apergillus fluvus	Oval	On air
Aspergillus sp.	Columnar, Quadrilateral transparent	On air
Bipolaris sorokiniana	Acicular branched	*Triticum aestivum*
Cladosporium $sp_{1.}$	Rosette- stellate, spindle shape stellate	On air
Cladosporium $sp_{2.}$	Radiated, Rosette- stellate, irregular	On air
Cladosporium $sp_{3.}$	Triangular	On air
Colletotrichum gloeosporioides	Radiated-stellate	*Datura metel*
Colletotrichum orbiculare.	'Fish-tail'	*Gerbera* sp.
Curvularia $sp_{1.}$	Triangular , Rosette	*Oryza sativa*
Curvularia $sp_{2.}$	Columner	*Houttuynia cordata*
Fusarium sp_1.	Granular	On air
Fusarium sp_2.	'Fish-tail'	*Solanum melongena*
Paecilomyces sp.	Bar, spindle shape stellate	On air
Penicillium sp.	Columnar	*Citrus sinensis*
Pestalotiopsis guepinii	Rosette- stellate	*Houttuynia cordata*
Pesatalotia sp.	Rosette- stellate, Quadrilateral stettate	On air
Phaeoisariopsis personata.	Rosette- stellate	*Arachis hypogaea*
Rhizopus sp.	Radiated-stellate	On air
Trichoderma viride.	'Fish-tail'	On air
Sterile fungi	Acicular, Columnar, Granular, Rosette-stelleate, Quadrilateral, Quadrilateral stellate,	*Datura metel*, on air

Eighteen different types of crystallographic structures were recorded and classified in this study. The types were Oval, Radiated, Radiated- stellate, Rosette, Rosette- stallate, Colamnar, Granular, Bar, Spindle, Spindle–stellate, Quadrilateral stellate, Quadrilateral, Triangular, Quadrilateral transparent, Irregular, Acicular, Acicular branched and Fish-tail. Present observation supports the results of Benedek (1961 and 1962) . In earlier studies crystals were reported to form in fungi grown in malt extract. In the present study all the crystal types formed on PDA medium at the temperature 25 to 28^{o} C and pH 6.0.

Oval, Acicular branched, Quadrilateral transparent, Granular, Quadrilateral stellate and Triangular shaped of crystals were not reported earlier, thus this is the first report of these six morphological types of crystals. Previously crystals had been reported in cultures of *Aspergillus flavus*, *A. fumigatus, Penicillium* sp,, C*urvularia* sp. and *Fusarium* sp. No report is available on the presence of crystals in cultures of *Aspergillus niger*, *Aspergillus* sp., *Bipolaris sorokiniana, Cladosporium* spp., *Colletotrichum gloeosporioides, Colletotrichum orbiculare, Pestalotia* sp., *Pestalotiopsis guepinii*, *Paeciliomyces* sp.,*Phaeoisariopsis personata*, *Trichoderma viride* and seven sterile fungi. Thus the results obtained from the present investigation indicates that

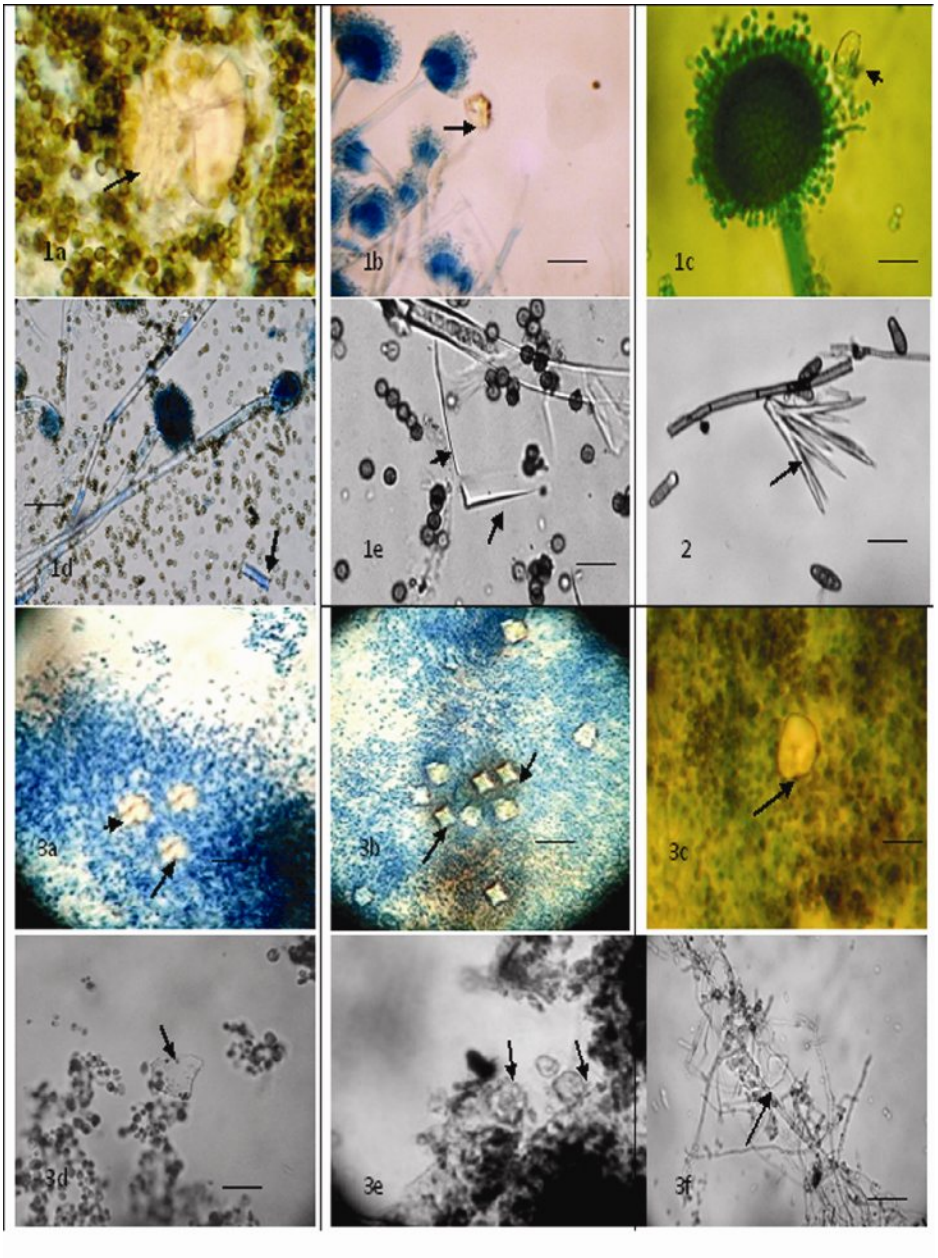

Plate 1. Formation of crystals in culture of fungi: 1. *Aspergillus niger*. 1b-c. A*spergillus fluvus*. 1d. *Aspergillus* sp. 2. *Bipolaris sorokiniana*. 3a-b. *Cladosporium* sp_1. 3c-e. *Cladosporium* sp_2. and *Cladosporium* sp_3. (Bar = 50 µm).

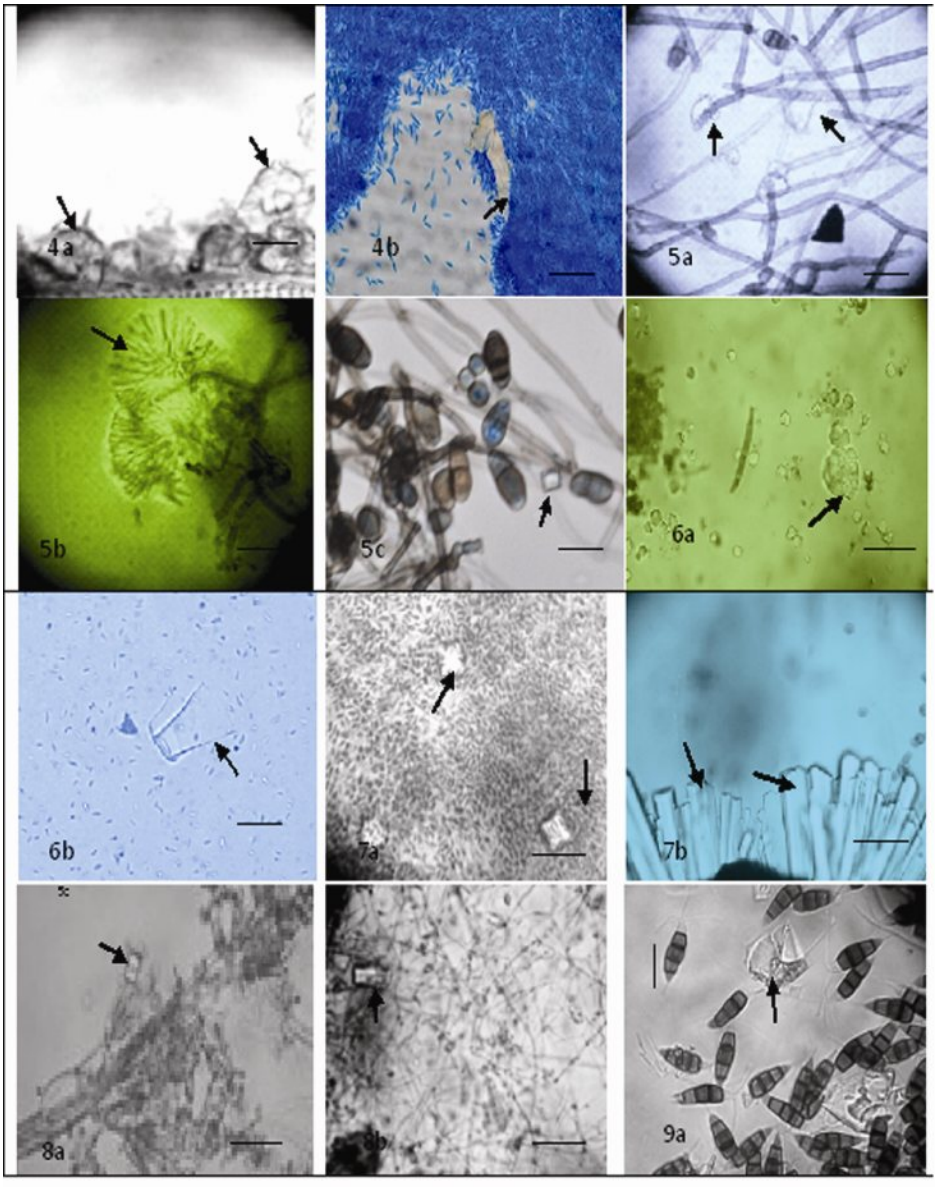

Plate 2. Formation of crystal in culture of fungi: 4a. *Colletotrichum gloeosporioides* . 4b. *Colletotrichum orbiculare.* 5a-b. *Curvularia* sp_1. 5c. *Curvularia* sp_2. 6a. *Fusarium* sp_1. 6b. *Fusarium* sp_2. 7a-b. *Paecilomyces* sp. 8a-b. *Penicillium* sp. 9a. . *Pestalotiopsis guepinii* (Bars = 50 µm and 100 µm [9a]).

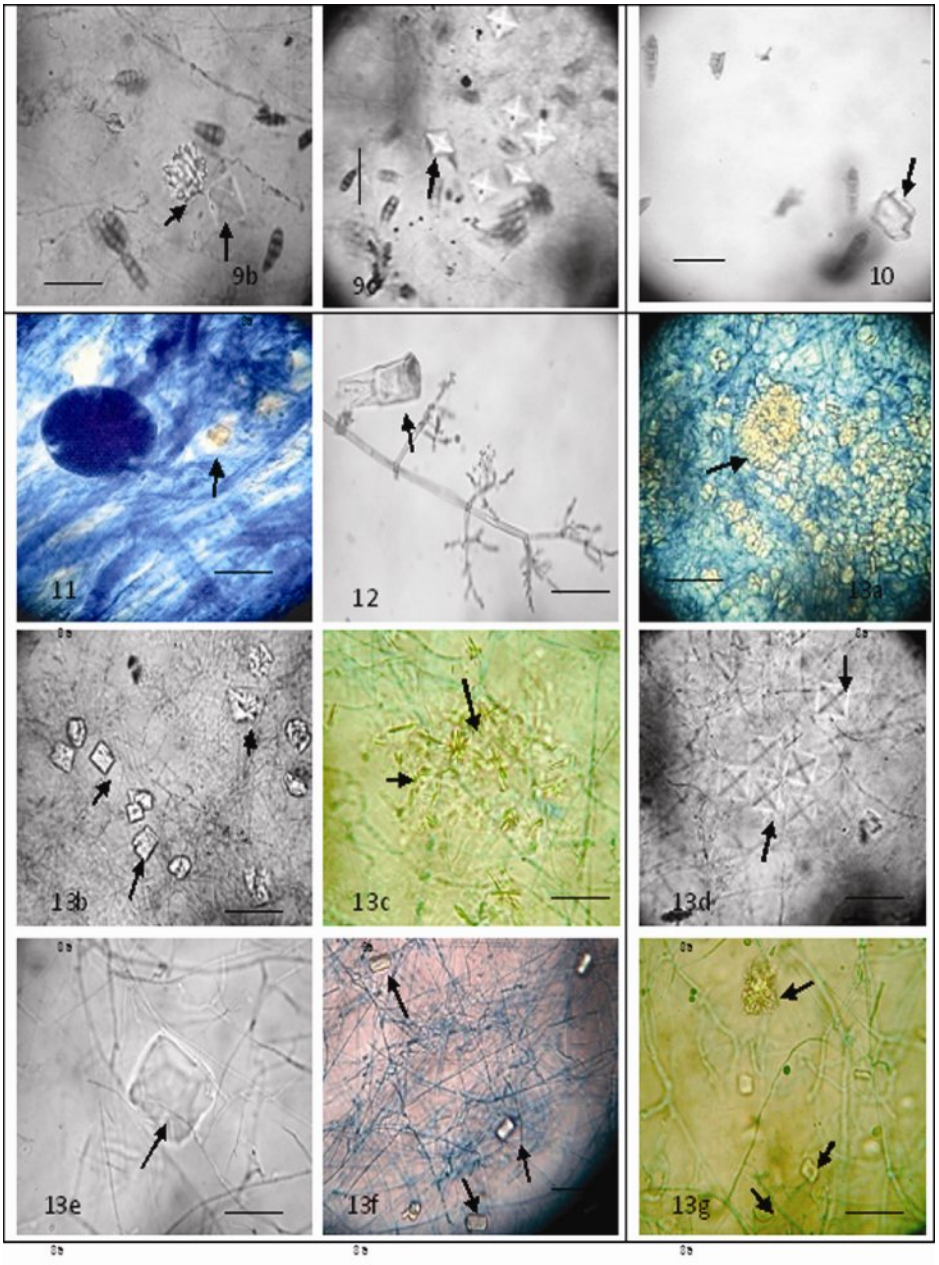

Plate 3. Formation of crystal in culture of fungi: 9b-c. *Pesatalotia* sp_1. 10. *Phaeoisariopsis personata*. 11. *Rhizopus* sp. 12. *Trichoderma viride*. 13a-g. Sterile fungi (Bar = 50 µm).

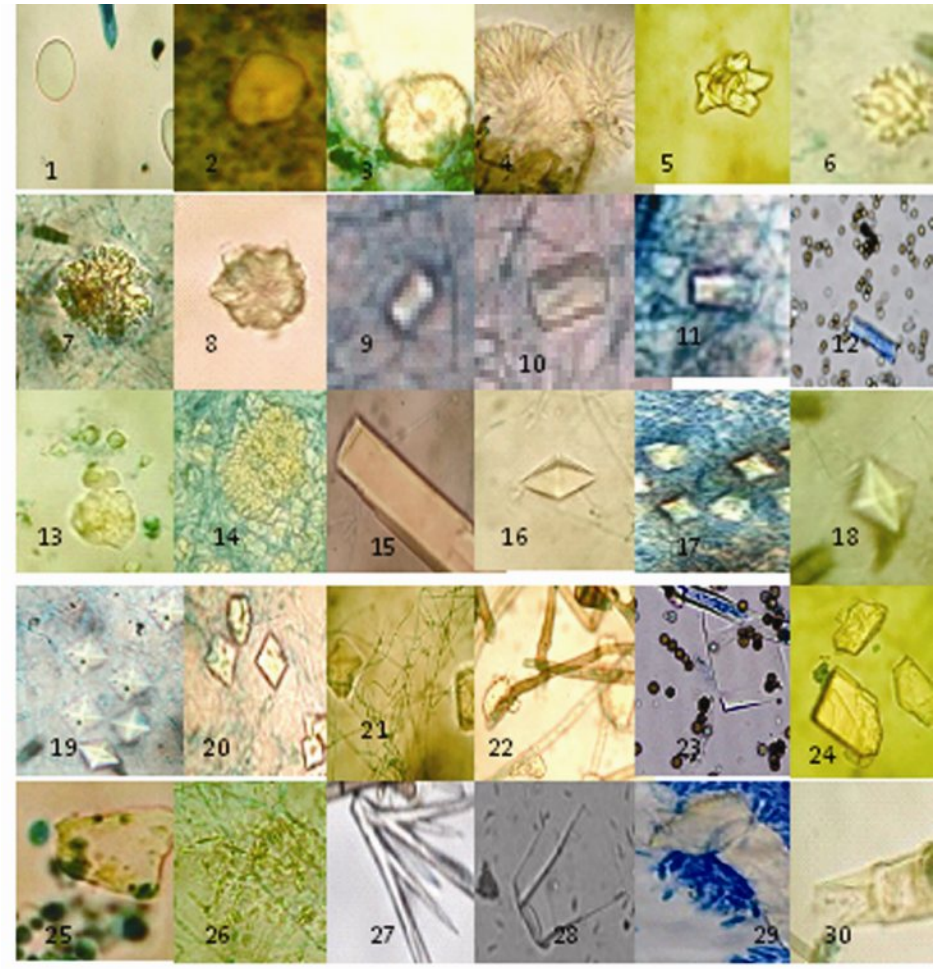

Plate 4. Different shapes of crystal: (1) Oval, (2) Radiated; Radiated-stellate (3) Rosette (4) Rosette- stellate (5, 6, 7, 8) Columnar (9, 10, 11, 12) Granular (13, 14), Bar (15) Spindle shape (16) Spindle stellate (17) Quadrilateral stellate (18-19) Quadrilateral (20, 21) Triangular (22) Quadrilateral transparent (23) Irregular (24, 25) Acicular (26) Acicular branched (27) 'Fish-tail'(28 - 30).

formation of crystals in the above mentioned genera are new record. After Benedek (1961 and 1962) no research report is available on crystal shapes found in fungal cultures. Mostly research had been carried out on chemical properties of crystals obtained from fungal cultures (Gadd 1999, Jarosz-Wilkolazka, and Gadd 2003 and Verrecchia *et al.* 1993). In Bangladesh contest present findings are new addition in Mycological Research.

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(Received revised manuscript on 27/4/2015)