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Research Paper

First report of presence of large unusual conidia in the infected parts of *Amomum subulatum* Roxb. and establishment of its identity

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ABSTRACT
Amomum subulatum Roxb., commonly known as Large Cardamom or Black Cardamom is an important cash crop of Himalayan region of Indian sub-continent. The farmers of hilly region of India, Nepal and Bhutan largely depend on this crop for their livelihood. However, due to the advent of highly virulent fungal disease in addition to pre-existing two viral diseases, the production has declined considerably. The fungal pathogen, C. gloeosporioides has been identified as main causal organism of blight disease in Large Cardamom based on isolation from infected parts and their morphological and molecular characterization. However, the microscopic exploration of infected parts, direct isolation of fungal sexual and asexual spores/conidia, their quantification and establishment of their origin has not been carried out. This study revealed the presence of large size unusual conidia not reported earlier in the infected parts of hardy region of crops such as leaf vein, sheath, rhizome etc in considerable quantity. Further, culture of these conidia shows the characteristics of C. gloeosporioides. This indicated that such unusual large size conidia were formed by C. gloeosporioides inside the hardy region of plant. It further established that the said organism is the main causal organism of blight disease in large cardamom.

Key words: *Large cardamom, blight disease, conidia, spore*

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I. INTRODUCTION

*Amomum subulatum*Roxb., belonging to the family Zingiberaceae is an important cash crop of Himalayan region of Indian sub-continent. It is mainly grown in hilly states of India, Nepal and Bhutan. The plant produces capsules encapsulating aromatic seeds which are mainly used as spice and flavoring agent in food and confectioneries industries. It is a sciophyte and loves to grow under the shade tree near the perennial streams in humid areas. The plant is rhizomatous shrubs which produces leaves from the extended overlapping sheaths.

In due course of cultivation process and area expansion, number of fungal disease infected the Large Cardamom crop in addition to the pre-existing two viral diseases. Some fungal species has developed high virulence and devastated the crop considerably. The main reason of such infestation is attributed to the weakening of plant due to cultivation of crop in unsuitable, dry and open areas. *Colletotrichum gloeosporioides* has been identified as main causal organism of blight disease in Large Cardamom and considered to be responsible for the devastation of crop and decline in production. The disease first appeared, as per farmers' representation, in Thoday-Tangta under Kalimpong sub-division of Indian state of West Bengal (Saju, 2010). It was first reported that the leaf blight of Large Cardamom is caused by *Colletotrichum* state of *Glomerella cingulata* (Stoneman) Spauld. & SchrenK) from kitchen garden of Muzaffarpur, Bihar (Prasad et al., 1984). It was, later, described as anthracnose caused by *G. cingulata*, perfect state of *C. gloeosporioides* from Sikkim, the traditional area of crop origin and cultivation (Srivastava, 1989). The fungal disease causing collar rot (*Fusarium oxysporum*), leaf streak (*Pestalotiopsis versicolor* and *P. royenae*) and leaf rust (*Phakospora elettariae*) of lesser prevalence has also been reported.

New pathogenic fungus *Epicoccum nigrum* was found to be associated with Large Cardamomthrough morphological and molecular characterization (Gurung, 2020). Some other pathogenic fungus recorded were *Pestalotiopsis maculans, Verticillium lecani, Curvularia eragrostidis, Colletotrichum gloeosporioides* and *Phoma carva* (Gurung, 2020). It has been observed that among the six varieties of *A. subulatum* grown in Sikkim, *Varlangey, Sawney, Ramla* and *Ramsey* were found to be blight infected (Gurung, 2020).

The microscopic exploration of infected parts of the Large Cardamom has not been carried out. The quantification of the sexual/asexual spore present inside the infected parts and their origin has not been established. The details of microscopic exploration, isolation and quantification of sexual/asexual spore, culture and isolation, morphological and molecular identification, establishment of main causal organism based on quantified data is described in this paper.

II. MATERIALS AND METHODS

MICROSCOPIC OBSERVATION

The infected parts of the plant including leaves, sheath, rhizome, capsules were collected from different locations. Thin sections were made from the infected leaf vein, sheath, rhizome and seed capsule. They were stained with lactophenol cotton blue and observed under compound microscope (Leica DM 1000). The size of the conidia was measured with the help of scale mounted in the microscope.

ISOLATION OF CONIDIA

The conidia from the infected parts of the plants mainly leaf vein, sheath, rhizome and seed capsule were isolated by grinding, sieving and centrifugation. Firstly, 200 gm each of infected parts were weighed and cut into small pieces of 20-25 cm long. It was then grinded finely in mortal and pastel and then followed by grinding in mixer grinder with 200 ml of water for 3 minute at high intensity. The finely grinded material was sieved through nylon mesh of 60 um pore size. The debris was washed with 200 ml water and filtered through same nylon mesh. The filtrates were allowed to stand in 500 ml beaker for 2 hrs for settlement. After 2 hours, the supernatant was carefully removed with the help of a small mason's level pipe without disturbing the pellet at the bottom. The pellet was transferred to oakridge tube and centrifuge at 5000 rpm for 15 minutes. The supernatant is discarded and the pellet is removed and place on the petriplate. It was allowed to dry at low temperature in oven (50^{o}C) for 6 hours. After the samples were fully dried, it was weighed and data recorded.

CULTURE OF ISOLATED CONIDIA

The small quantity of conidia of about 1 gm in the form of white powder after isolation as above was surface sterilized with 4% sodium hypochlorite. The sample was placed inside 5ml gama irradiated falcon tube containing sodium hypochlorite. It was centrifuge at 3000 rpm for 7 minutes. The supernatant discarded and sterile distilled was added. The tube was rotated upside down and again centrifuge at 2000 rpm for 5 minute. This process was repeated for two more times to ensure complete removal of sterilent. The conidia were then cultured on Potato Dextrose Agar in petriplates. It was incubated at 28^{o}C. After 20 days, mycelium appeared. It was further sub-cultured in fresh medium and observed microscopically at regular interval of two days.

III. RESULTS AND DISCUSSION

Microscopic observation of infected part of Large Cardamom plants including leaf veins, sheath, rhizome and fruit capsule reveal the presence of unusual large size conidia in considerable quantity, which was not reported earlier. The size ranges from 15 to 25 um in length and 6 to10 um widths. The shapes vary from truncate, ovoid, fusiform, sub-globose to oval (Fig. 1A-1C).

Fig.1A: Isolated sheath conidia

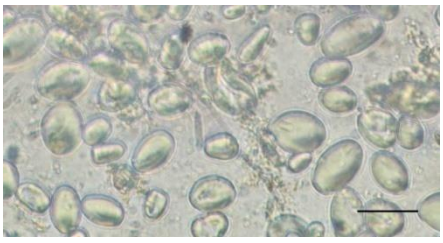

Fig. 1B: Microscopic view of sheath conidia (Bar=20um)

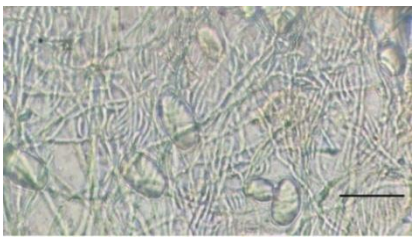

Fig.1C: Sheath conidia formed in cultures (Bar=20 um)

The quantification of isolated conidia from different infected parts of various level of infection indicated the highest quantity of 5.26 gram of conidia per 200 gm sample was found in the severely infected

sheath followed by rhizome of same severity. The lowest quantity of conidia was isolated from the dead leaf veins. Interestingly, the quantity of conidia decreased in dead plants as compared to mild infected and severely infected one (Table 1 & Fig. 2)).

Table 1: Results showing the quantity of isolated conidia from different infected parts of the Large Cardamomplant in gram per 200 gm sample.

	Leaf veins	Sheath	Rhizome	Fruit capsule
Healthy	0.88	1.43	1.05	0.38
Mild infected	1.58	2.39	2.02	1.37
Severely infected	1.88	5.26	4.80	2.55
Dead	0.36	1.92	1.88	-

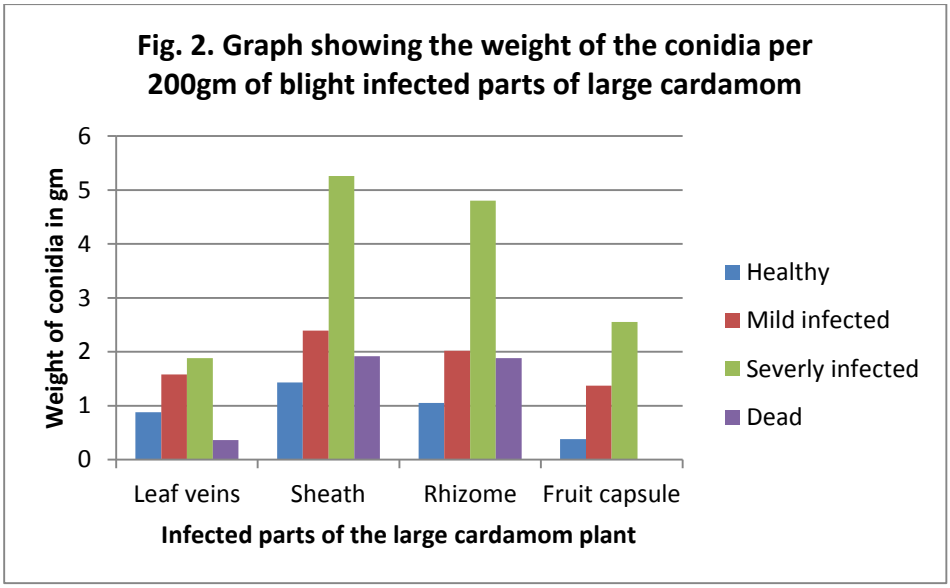

Table 2: Cultural and microscopic characteristics of conidia isolated from infected parts of Large Cardamomand its identification

Colony characteristics	Mycellium	Spore	Conidia	Morphological identification
Cottony white fluffy, light orange concentric ring, dark green centre	Septate	Plenty of cylindrical spores with round end of about 12um length and 6um width	Conidia inter-calary, 15-25um length & 6-10um width truncate, ovoid, fusiform, sub-globose, oval, hyaline	*C. gloeosporioides*

The culture of the isolated conidia shows the characteristics feature of *C. gloeosporioides*, the main causal organism of blight disease in Large Cardamom (Table 2). Interestingly, similar type of large size conidia also formed in the cultures entangled with mycelium in 25 days old sub-cultured culture. This indicated that such conidia is being formed by *C. gloeosporioides*, the main causal organism of blight disease in Large Cardamom in the hardy region of plant such as leaf vein, sheath, rhizome and fruit capsule in large quantity. It also indicated that *C. gloeosporioides* is the main organism responsible for devastation of crop.

IV. CONCLUSION

The presence of large size conidia in the hardy parts of Large Cardamom plant i.e. leaf vein, sheath, rhizome and fruit capsule in considerable quantity and establishment of its origin as *C. gloeosporioides* indicated that this organism is responsible for the major devastation of Large Cardamom crop.

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