

# FIRST REPORT: ASSOCIATION OF *FUSARIUM INCARNATUM* WITH RHIZOME ROT OF *ZINGIBER OFFICINALE* ROSCOE IN SIKKIM

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**ABSTRACT:** Ginger is a third economically important crop grown after large cardamom and citrus in Sikkim. The crop is susceptible to number of pests and disease, the most common disease being rhizome rot caused by *Fusarium spp.* and *Pythium spp.* which destroys the yield during cultivation as well as in storage. Farmers are incurring severe losses due to pest and disease for the past many years. However, there is a need to gather more information about pathogens related to rhizome rot, which provides a way to search for more effective prevention and treatment strategies. Infected ginger rhizomes were collected from different parts of Sikkim. The present study reports the association of *Fusarium incarnatum* (Desm.) Sacc. (synonym: *Fusarium semitectum*) with rhizome rot of ginger. Morphologically it is floccose with abundant powdery aerial mycelium and brown pigmentation, 3 to 4 septate heterogenous macroconidia of 20 µm. Pathogenicity test revealed 38.48% PI value causing moderate rhizome rot. This is the first record of ginger rhizome rot caused by *F. incarnatum* in Sikkim. With this report crop protection will certainly be strengthened and a potential component for integrated disease management to prevent and control rhizome rot in ginger field could be suggested to the farmers.

**Keywords:** *Fusarium incarnatum*, *Zingiber officinale*, rhizome rot, Pathogenicity, Sikkim.

## I. INTRODUCTION

*Zingiber officinale* Roscoe (2n=2x=22, Zingiberaceae), the largest family of order Zingiberales is a perennial monocotyledonous herb, comprised of 47 genera and 1400 species (Hogarth, 1999). It is a valuable spice crop and plays important role in Indian Ayurveda as a folk remedy to cure nausea, loss of appetite, motion sickness, cold and flu, inflammation etc. In local markets ginger is usually sold as raw ginger but there are other valuable products like dry ginger, ginger powder, ginger oil, and oleoresin (Kizhakkayil and Sasikumar, 2011). India has the largest area under ginger cultivation accounting 49% of ginger grown area and 72% of its production (Ravindran and Babu, 2005). It is an important cash crop in Northeast region (NER), more than 50% of total ginger production takes place in North East and Uttarakhand (Rahman et al, 2009). Sikkim ranks 3<sup>rd</sup> in area and 5<sup>th</sup> in production (Rahman et al, 2007). NER ginger cultivars are high rhizome yielders with higher oil and oleoresin content compare to ginger from other parts of India (Spices Board, 2007, Yadav et al, 2004).

Ginger is vulnerable to numerous pest and diseases (Srivastava et al, 1998). Earlier researchers reported 39 potent pathogens which includes bacteria, fungi, and virus causing diseases like soft rot, basal rot, yellows, bacteria wilt, leaf spot, banded leaf blight, chlorotic fleck virus, mosaic disease, dry rot in the field or in storage (Gupta and Manisha, 2017). Among them most lethal disease for ginger is fungal diseases. Various workers reported different fungal diseases viz soft rot or rhizome rot or *Pythium* rot caused by *Pythium spp.*, yellow disease by *F. oxysporum* f. sp. *Zingiberi*, *Phyllosticta* leaf by *Phyllosticta zingiberi*, *Colletotricum* leaf spot by *Colletotricum zingiberi*, Thread blight by *Pellicularia filamentosa*, Storage rots by *Fusarium oxysporum*, *Pythium deliense* and *Pythium myriotylum* (Gupta and Manisha, 2017, Dake, 1995). Other species of *Fusarium* such as *F. solani* (Mart.) Sacc., *F. equiseti* (Corda) Sacc., are also found to be associated with disease of ginger rhizomes (Rosenberg, 1962). These fungal diseases are serious concern in most ginger growing areas because their infection leads to total crop failure. Pathogens are mostly soil inhabitants possessing high degree of competitive saprophytic ability. The infection spreads easily through soil, rain water or irrigated water in the field (Dake, 1995).

Ginger is one of the main cash crop of Sikkim and its loss on production will directly hamper small and marginal farmers. The survey during the study revealed that pest and disease being the main factor obstructing the production and limiting yields. The declining yield of ginger has led to the decrease in sustainable economy of the

farmers. Furthermore, there is need of detailed study of pathogens and appropriate remedial measures and its control, which could rejuvenate the cultivation of ginger.

In this investigation, rhizome rot has been found associated with *Pythium spp.*, *Fusarium oxysporum* along with *Fusarium incarnatum* (Desm.) Sacc. which was earlier reported to affect crops and vegetables like rice, sorghum, wheat, barley, maize, long bean, bitter gourd, cucumber, green chili etc. (Ebadi and Riahi, 2013, Latiffah et al, 2013). *Fusarium semitectum* along with *Nigrospora sphaerica* was also reported to cause ginger rhizome rot in Brazil (Moreira et al, 2013). According to earlier report, soft rot was predominant in state followed by bacterial wilt (Rahman et al, 2007). On the basis of our knowledge, this is the first report of this particular *Fusarium* species infecting ginger in Sikkim.

## II. MATERIALS AND METHODS

### 2.1. Study area

Samples were collected from different ginger grown fields of all four districts of Sikkim viz. East (Dugalakha, Pachey, Dalapchand, Noapgoan, Rongli), West (Pakkigoan, Lingchom, Tikzek, Chakung, Gelling), South (Mani ram, Tarku, Saddam, Bermoik, Namthang), North (Passingdang, Lingthem, Mangshilla, Ringhim, Phodong) as shown in the Figure 1.

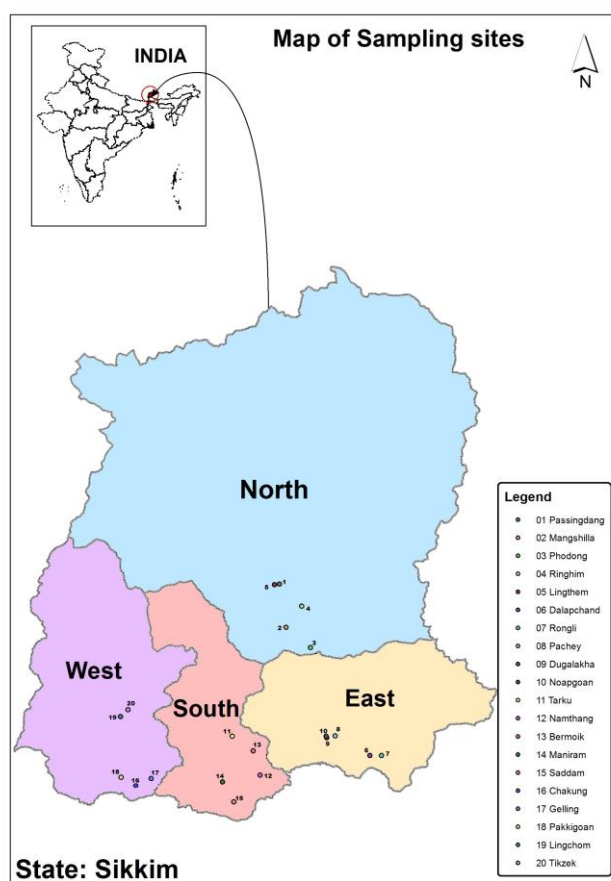
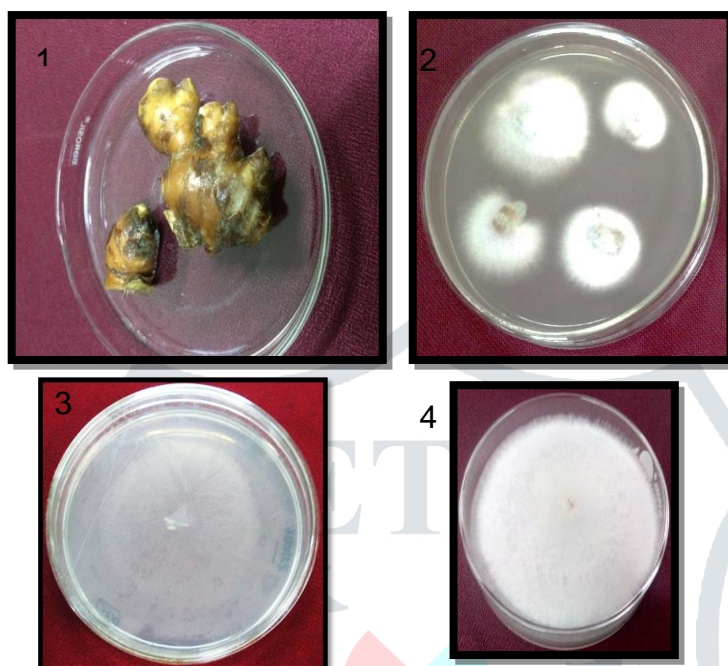


Figure 1: Map showing sample collection sites

### 2.2. Sample collection and Isolation

Samples (diseased rhizomes) were collected from various locations of ginger cultivation fields during the month of July-August (maximum infection period). The rhizome samples were transferred into sterilized polybags (zip-lock) and brought into Laboratory at Sikkim State Council of Science and Technology. The samples were

cleaned with running tap water to remove all soil and dirt particles followed by washing with double distilled water. Next, rhizomes samples were cut into small pieces (3-5 mm) in length. Accordingly, samples were surface sterilized by 0.5% sodium hypochlorite for 3 minutes, blotted dry on sterile filter paper and placed onto different media like water agar (WA) and potato dextrose agar (PDA) and incubated at 28-30 °C. Mycelia growth was observed after 4 to 5 days, hyphal tips were transferred to PDA slant to obtain pure culture and maintained on PDA at 4°C (Trikarunasawat, 2008).



**Figures 2: Isolation of pathogenic fungi (1-2 Infected ginger rhizome and Isolation of pathogens, 3-4 Pure culture of isolated fungi on WA and PDA plate)**

The fungal isolate was observed morphologically (colony morphology and pigmentation) and microscopically (shape, size, type and manner of conidia formation, production of microconidia, and macroconidia) after 14 days (Leslie and Summerell, 2007). Furthermore, for the identification of the fungus, isolate was sent at Agharkar Research Institute, Pune, India.

### 2.3. In vitro Pathogenicity test

Pathogenicity test was evaluated following method of Ayele (2015) with some modifications. Fresh and healthy rhizomes were washed in sterile distilled water and surface sterilized using 1% sodium hypochlorite and then washed thrice with sterile distilled water. By using 5 mm diameter sterile cork-borer, holes were dug, the plug was pulled out and 1ml spore suspension of *Fusarium incarnatum* ( $1 \times 10^6$  spores) was injected at the bottom of the hole on the rhizome. In order to prevent extraneous infection, the wounded areas were capped with about 5 mm thick sterile agar discs. Inoculated rhizomes were then placed in air tight bottle and weighted. Weighted bottle were kept moistened by adding 3 ml of sterile distilled water onto filter papers placed beneath the rhizome and incubated for 15 days at  $30 \pm 10$  °C. Five rhizomes were used for pathogenicity test per each isolate. Control was sterilized distilled water of equal volume placed into the holes of healthy rhizome. The rhizomes were reweighed at the end of 15 days to determine the pathogenicity index using the expression below:

$$\text{Pathogenicity index (PI)} = 100 - [WD \times 100] \div WH$$

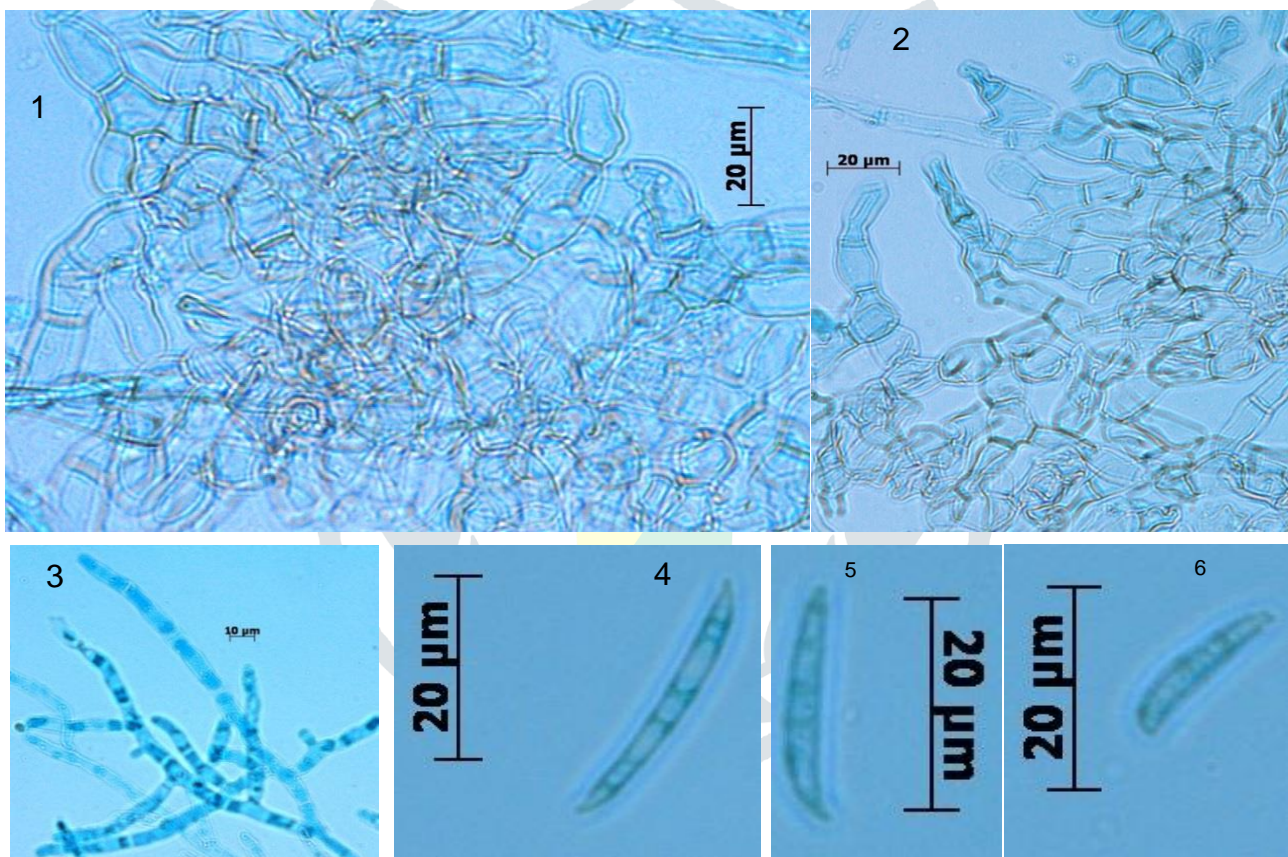
Where: WD= weight of the diseased rhizome and WH= weight of healthy rhizome.

Observation was made by cutting the rhizome with sterilized scalpel along the plane of inoculation. For confirmation, if the rotting of the rhizome was due to the inoculated pathogens, fungus grown was inoculated on to PDA.

### III. Results

The pathogenic fungus was identified as *Fusarium incarnatum* (Desm.) Sacc. which belongs to family *Nectriaceae*. The colony was floccose and powdery. Abundant aerial mycelia, white to light beige in appearance were observed. Initially mycelia were off white and with age it becomes brown along with brown to dark brown pigmentation on reverse side of the colony. Macroconidia were heterogeneous, straight to slightly curved, the apical end uniformly tapering, 3 to 4 septa of 20  $\mu\text{m}$  (Figures 3:4-6). These are characteristics consistent with *Fusarium incarnatum*.

The pathogenicity test resulted in PI value of *F. incarnatum* to 38.48 % indicating it has the potential to induced rhizome rot. It caused moderate rot of test rhizome after 8 days of inoculation without exudation of liquid (Nishijima et al, 2004). The internal part showed pale to brown discoloration after transverse section. The *Fusarium* isolate showed cottony mycelial growth over the surface of the inoculated rhizomes whereas no fungus growth was seen on control rhizome. Fungus was re-isolated from the infected rhizome and identified morphologically, thus satisfying the Koch's postulates.



**Figures 3: Morphology of *Fusarium semitectum* (Source : Ginger rhizome)**  
(1-2 Thick modified vegetative hyphae, 3 Branched simple hyphae, 4-6-Heterogenous macroconidia).

### IV. Discussion

*Fusarium incarnatum* is widely distributed in soil as saprophytes or soil inhabitants (Burgess, 1988, Leslie et al, 1990). They are reported to cause several diseases such as canker of walnut, pod rot, seed rot and root rot of beans, corky dry rot of cantaloupe, storage rot of banana and wilting of alfalfa along with crops such as sorghum, rice, wheat, barley and maize of subtropical and temperate regions (Zaccardelli, 2006, Seta et al, 2004, Griffee, 1971). It is a common fungus in cereal fields. Several seedling diseases are found to be frequently related to *Fusarium incarnatum* and reported to be the major cause of seedling death in some countries (Zhang et al, 1996). Even though, this fungus is frequently found involved in various plant diseases, it is not considered in category of important plant pathogens (Ebadi and Riahi, 2013).

*Fusarium spp.* which infects ginger rhizome in Sikkim is *Fusarium oxysporum* f.sp. *zingiberi*, *Fusarium solani*, *Fusarium equiseti*, *Fusarium moniliforme*, *Fusarium gramineacearum* and *Fusarium roseum* (Srivastava, 1995). Similar occurrence of *Fusarium incarnatum* in ginger field was reported by researchers from Brazil including many other fungi and bacteria (Moreira et al, 2013). The susceptibility of ginger to this particular fungus may be due to variable environmental conditions, the nutrient deficiency or imbalance between soil moisture and oxygen which usually make plants prone to pathogens (Kennelly et al, 2012). It could also act as one of the opportunistic pathogens. With the potential effect of climate change, environment factors such as temperature, rainfall, relative humidity, soil moisture, pH, soil types and soil fertility are changing making crops more vulnerable to pathogens. This is the first report of *Fusarium incarnatum* associated with rhizome rot of ginger in the State. Ginger is a high value crop, therefore, extensive study is required to provide new insight to the disease and its technical hitches. It is anticipated that this particular study will help combating rhizome rot in Sikkim and further developing new management strategies. However, molecular analysis would be required to characterize *Fusarium incarnatum* to confirm its taxonomy.

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