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Stem Rot, a new disease of *Valeriana jatamansi* in the Sub-Himalayan zone of Darjeeling district of West Bengal, India

S.BASKEY[1], S.HEMBROM[2], S. Ali [3], B.R.SHARMA[4], B.Tudu[5] AND S.CHAKROBORTY[6]

[1234] *Department of Plant Pathology,UttarBanga Krishi Viswavidyalaya, Regional Research Station (Hill Station)Kalimpong , Darjeeling-734301, West Bengal, India.*

[5] *Department of Agril. Entomology,UttarBanga Krishi Viswavidyalaya, Regional Research Station (Hill Station)Kalimpong , Darjeeling-734301, West Bengal, India*

[6]*Department of Plant Breeding, UttarBanga Krishi Viswavidyalaya, Pundibari, Coochbeher-736165, West Bengal, India*

Abstract

A new stem rot disease is found to occur naturally on Valeriana jatamansi plants in greenhouses at Hill Campus of U.B.K.V. Kalimpong,Darjeeling. In order to identify its pathogen, we conducted a fungal series of isolation and purification, plant reinoculation, and ascus and ascospore induction from the sclerotia. The isolate caused typical water-soaked lesions after reinoculation and produced sclerotia both on Valeriana plants and culture medium plates, and the sclerotia could be induced to produce discal apothecia and 8 binucleate ascospores per ascus. These disease symptom and fungal morphology data revealed that the fungus Sclerotinia sclerotiorum (Lib.) de Bary was the pathogen for valeriana stem rot. Taking all these data together, we concluded that the fungus that caused the valeriana stem rot is S. sclerotiorum (Lib.) de Bary. This is the first report that valeriana jatamansi is naturally infected by S. sclerotiorum.

Key Word: Medicinal plant, Valeriana jatamansi, Stem rot, Sclerotinia sclerotiorum ,Disease, Identification

I. INTRODUCTION

Valeriana jatamansi (Family: Valerianaceae), distributed in temperate Himalayas (1500to 3000 m) is valued both in traditional and modern systems of medicine, is one of the most important medicinal plant in the sub-Himalayan zone of Darjeeling district of West Bengal,India. Traditionally it is used for treating hysterical fits, nervous unrest, emotional troubles, epilepsy, asthma, leprosy, etc. The roots are also used as carminative, laxative, antiperiodic, hypnotic and aphrodisiac. Most of the therapeutic properties like tranquillising and sedative effects of this species are due to the presence of a group of compounds termed as valepotriates (present in roots and rhizomes) and essential oils (present mostly in roots). This species becomes extinct due to overexploitation of this species and Low germination percentage, viability of seeds, long gestation periods and delicate field-handling are some of the factors which discourage commercial cultivation and created lot of pressure on its natural resources which has led to considerable depletion of it stock , IUCN describes this species as globally vulnerable (Mukherjee *et.al.,*2013). Besides this, during the monsoon season (June-august) severe stem rot of Indian Valeriana was observed in different experiment fields in Regional Research Station, Kalimpong, Darjeeling, under the aegis of Uttar Banga Krishi Viswavidyalaya, West Bengal, India and in the major growing areas of valeriana. Havoc loss has been recorded in the infected fields as high humidity along with high fog intensity aggravated the rotting of the plants. Hence, the present investigation deals with the detection and identification of the pathogen *Sclerotinia sclerotiorum* from the infected plant of *valeriana jatamansi* .

II. MATERIALS AND METHODS

Sample Collection

The infected stem showing typical external symptoms like water-soaked spots, white cottony growth of mycelium developed on the lesion and wilt like plants (Fig.1) were collected for identification of causal agent during the roving and fixed plot survey of 2011-14 from farmer's field of major *valeriana jatamansi* growing areas of Darjeeling district in West Bengal. The collected samples were transported to plant health clinic (PHC) in fresh condition in plastic bags and stored at 25^0C for 24 hours for further analysis.

Isolation of *Sclerotinia sclerotiorum* from infected *valeriana jatamansi* stem

The fungus was isolated by taking small pieces of infected stem were cut aseptically from the edge of typical lesion with a little portion of healthy tissue. The infected stem bits were surface sterilized in 70% alchohol or 1% sodium hypochloride and washed in three series of sterile water to remove traces of alchohol. Then, after slightly air drying, were poured onto the sterilized petriplates containing PDA media. The inoculated plates were incubated at 25^{oC} for 72hours. Observations were made for development of mycelia growth on PDA media.

Purification of Fungal Culture

The suspected fungal colonies were picked up with the help of sterilized inoculating needle and place onto the surface of sterilized petriplate containing PDA media .The inoculated plates were incubated at 25^oC for 48 to 72 hours and the observation made for the development of fungal colonies. The purified fungal colonies were placed on PDA slants and store at 5^oC in refrigerator for future use.

Identification of Causal organism

Sclerotinia sclerotiorum (Lib.) de Bary is a facultative parasitic Ascomycete fungus (Kirk *et al*., 2001) and can grow well even in an unfavourable environment and survive for up to 8 years in soil in the sclerotial form (Adams and Ayers, 1979). It can infect as many as 408 plant species including many important crops, such as rapeseeds and soybean, and many vegetables (Bolton *et al*., 2005). It causes water-soaked lesions on the leaves or stem rot in stems of some infected plants. The most obvious symptoms of plants infected by *S. sclerotiorum* are necrotic tissues covered with patches of fluffy white mycelia and sclerotia are produced after mycelial growth when the nutrition is not sufficient or other conditions are favourable for sclerotial development (Christias and Lockwood, 1973). Sclerotia play an important role in disease cycles as they are the primary structures for their long-term survival and produce inoculums for further infection. Sizes of sclerotia are dramatically different depending on their host. Sclerotia germinate either carpogenically or myceliogenically, resulting in two distinct categories of diseases under different environmental conditions. Hyphae developed when sclerotia germinate myceliogenically and can directly attack plant tissues under soil (Le, 1979). However, apothecia are produced when sclerotia germinate carpogenically and ascospores can be projected to the air and infect aboveground portions (Smith *et.al.,* 1989). The morphological characteristics such as mycelial growth rate, shape, Size and colour of sclerotia and ascus & ascospore induction from the sclerotia were studied.

Pathogenicity on *valeriana jatamansi*

Pathogenicity test was carried out to find out whether the isolated fungal culture was capable of producing typical symptoms of stem rot under artificial inoculation condition on *valeriana jatamansi* plants or not. . The fungus *Sclerotinia sclerotiorum* was maintained in potato dextrose agar (PDA) culture and multiplied for 15 days on sterilized wheat grains at 25^oC and colonized grain served as the source of inoculums for artificial inoculation. Forty-day-old healthy plants were

inoculated with a few colonized grain through slight insertions in the intersections between the stems and branches (n = 10). Five plants were inoculated with sterile wheat grains. All inoculated plants were misted to runoff and bagged in plastic for 3 days to maintain high humidity. The bags were removed after 72 hours. Observations were made for the development of symptoms of stem rot. The isolated and re-isolated fungal culture was compared with the original culture of *Sclerotinia sclerotiorum* by studying colony morphology, colour and characters.

III. RESULTS AND DISCUSSION

Isolation of *Sclerotinia sclerotiorum* from infected Indian valeriana stem

The pathogen was isolated on potato dextrose agar (PDA) media. On PDA, the fungus grew very fast at a rate of 25-30 mm per day when incubated at 25°C.

Purification of Fungal Culture

We followed a standard procedure for fungal isolation and purification. Well separated out colonies isolated from the infected stem were purified by re-culturing on the surface of PDA media. The culture was stored on PDA slants at 5°C. These were kept as the stock cultures for further studies**.**

Identification of Causal organism

To conduct pathogen identification and analysis, the fungus was isolated from infected tissues of *Valeriana jatamansi* and cultured on PDA medium. The fungus started to produce white masses when growing to the edge of the Petri dish. The size of mycelium masses became bigger and their colours became darker as time proceeded. Finally, many black sclerotia formed (Fig.2).When the *Valeriana jatamansi* leaves were inoculated with the isolated fungus, the symptoms were the same as those under natural infection.

Sclerotial Germination and formation of apothecia

Sclerotia germination and apothecia formation were also studied; sclerotia were first incubated in moist sandy soil for 5-9 weeks at 5°C and then placed in the condition at 15-20 °C with scattered light. Sclerotia geminated quickly after 20 days. Stipes and receptacles of apothecia were formed at first, then their tops grew swollen and discal apothecia with cupped centres were formed. Hollowness in the centre became flatter with augmentation of the apothecia and the hymenial layer spread fully until asci became mature and ascospores emanated .The numbers of apothecia produced per sclerotium were not equal, ranging from only 1 to as many as 9-10.

Morphology study of asci and ascospores

Apothecia were cut into thin slices, then stained with trypan blue and observed with a microscope. The hymenial layers of the apothecia were found to be full of asci, each ascus containing 8 ascospores. Mature ascospores were released from the top of the asci .Many paraphyses could be seen among the asci . Each ascospore had two nuclei when stained by EB. The results suggest that ascospores were single binuclear cells, coinciding with the description in (Kohn, 1979).

Pathogenicity on Valeriana jatamansi

The conidia of *Sclerotinia sclerotiorum* artificially inoculated to 6 weeks age of Indian valeriana. The first symptoms of the disease observed 15 days after inoculation. The fungal infection process was observed. The fungus infected old leaves firstly and expanded rapidly through the petiole into the stem, causing water-soaked lesions. The upper leaves were subsequently infected until the whole plant finally died off. The disease was frequently observed to trans-infect other healthy plants if the infected leaves made contact with uninfected plants .The hyphae grew luxuriantly and

adjacent plants could also become infected with the disease through the hyphal growth when the atmosphere humidity reached 90% .Sclerotia formed on the surface of plants after plants had died (Fig.2). The fungal pathogen was consistently reisolated from inoculated plants. The pathogen was identified as *Sclerotinia sclerotiorum* (Lib.) de Bary on the basis of morphological characters**.**

The fungus we isolated could infect *Valeriana jatamansi*,causing water-soaked lesions in leaves, developing sclerotia on the surface of infected tissues and spreading across leaves in contact with each other at high moist conditions. These disease symptoms are the same as that caused by *S. sclerotiorum* (Lib.) de Bary in other plants. Mycelia of *S. sclerotiorum* (Lib.) de Bary in host plants or in culture look hyaline, septate, branched and multinucleate, and their colors changed from white to dark as melanin accumulates. It cannot produce conidiophore during the asexual period. Hyphae tend to form sclerotia (Kirk *et al*., 2001). These are also the characteristics of our isolate. Our further observation on sclerotia germination and apothecia production confirms that there are three stages during the course of sclerotial development (Chet and Henis, 1975; Boland et.al., 1994; Bolton *et al*., 2005). At initiation stage, hyphae aggregate to form a white mass, then further aggregate to increase the size of sclerotia; finally, surfaces of sclerotia are delimited, with melanin deposited in peripheral rind cells and interiors of sclerotia become consolidating. Each sclerotium can produce one or more apothecia consisting of a stipe and a receptacle with a flat to concave hymenial layer (3-9 mm in diameter). Asci are cylindrical sac-like zygote cells and are rowed in the hymenial layer. Each ascus contains eight hyaline, ellipsoid binucleate ascospores (5-6) µm × (10-11) µm. The morphology and development of our isolate were similar to those of *S. sclerotiorum* as described by Kohn (1979) and Wang et al. (2008). Taking all morphological, developmental data together, we confirmed that the fungus isolated from naturally infected *Valeriana jatamansi* plants is a necrotrophic fungal pathogen, a strain of *S. sclerotiorum* (Lib.) de Bary. The Indian valeriana plant is severely affected by fungal pathogen. It has become more serious in Indian valeriana growing areas of Darjeeling district as this medicinal plant cultivation is getting popular among the hill farmers. A critical problem in the study of fungal pathogen is the correct identification of the infectious agent. The study of diseases in medicinal plants is an important aspect of assessing plant health. Hence, in the present investigation the identification and isolation of pathogen from infected plants of *Sclerotinia sclerotiorum* has been demonstrated for the first time in West Bengal as well as India.

IV. CONCLUSSION

The experiments confirmed the presence of *Sclerotinia sclerotiorum* as causative pathogen for stem rot disease in the infected leaves of Indian valeriana. This result also indicates that the Indian valeriana plants grown in Darjeeling district of West Bengal is highly infected by the fungus.

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