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J. Mycopathol, Res, 55(4) : 365-368, 2018;
ISSN 0971-3719

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First report on occurrence of Leaf spot disease on *Hippophae salicifolia* D. Don caused by *Alternaria* species from Uttarakhand, India

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Received : 04.09.2017 Accepted : 15.09.2017 Published : 29.01.2018

During 2014-2015, occurrence of leaf spot disease was observed on *Hippophae salicifolia* D. Don plants at some selected sites in Uttarkashi, Uttarakhand, India. The typical disease symptoms were observed on the abaxial surface, tips and spiny margins of leaves. Disease spots were sunken, dry, necrotic, dark maroon to dark brown in color. On the basis of morphological and microscopic characteristics of the fungus, *Alternaria tenuissima* was found to be associated with the leaf spot disease. Koch's postulate was applied to confirm the causal organisms of the disease

Key words: Leaf spot, *Hippophae salicifolia, Alternaria tenuissima*, Uttarakhand

INTRODUCTION

The Indian Trans-Himalaya represents a distinct biogeographic zone, characterized by distinct cold arid ecosystem that is spread across Himachal Pradesh, Jammu and Kashmir, Uttarakhand and Sikkim. In cold desert environment natural resources are limited and only a few plant genera like *Hippophae* are adapted to cope up with the harsh climatic conditions. Genus *Hippophae* (Family-Elaegnaceae) represents 7 species and 8 subspecies worldwide (Swenson and Bartish, 2002). It is a dioecious or occasionally monoecious, spinaceous and arboresent shrub varying in height from 50 cm to 8 m. It is tolerant to extremes of temperature (-43 to +45^{0}C), resistant to drought conditions and well adapted to the salinity and alkalinity (Kumar, 2003; Jodha *et al.* 1992). It is supposed to be a store house of nutrients and vitamins and many items like jams, soft drinks, sauces, and pickles are being prepared. In Indian Himalayan region, sea buckthorn plant can offer benefits of nutrition, food, medicine, cosmetics etc. to the rural people for their socio-economic development. Sea buckthorn leaves are used for antioxidant and other properties. *Hippophae salicifolia* (Sea buckthorn) because of its multifarious benefits is called wonder plant or cold desert gold. Realizing the importance of sea buckthorn for ecological, social and economical development, a number of scientific studies have been undertaken in India (Chauhan *et al.* 2001;Singh *et al*. 2006; Dhyani *et al.* 2007; Butola and Badola, 2008; Singh *et al*. 2008; Singh *et al.* 2010), but a little work has been done regarding its pathological aspect.

Despite its therapeutic and antimicrobial potential, sea buckthorn is susceptible to various fungal diseases. Leaf spot is an important disease that not only affects the leaf texture but also reduce the quality of leaf that is used for medicinal and commercial purposes. Therefore, it was the objective of this study to identify the causal agent of leaf spot symptoms on *Hippophae salicifolia* D.Don.

MATERIALS AND METHODS

Sample collection and study of symptoms

Leaf samples were collected from different locations of Uttarkashi district. The studied populations included Dharali, Yamunotri, Hanuman Chatti, Sukhi, Harsil, Bhairon ghati and Gangotri in Uttarkashi district of Uttarakhand, India. Ten in-

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fected leaf samples were collected randomly from each site, placed in labelled sterile poly bags and taken back to laboratory to study for the presence of leaf spot diseases on *Hippophae salicifolia* during the flowering and fruiting period of 2014 and 2015. The morphology of the symptoms was studied with the help of hand lens and dissecting microscope.

Isolation and purification of pathogen from diseased leaves

Diseased leaves of *Hippophae salicifolia* were taken into the laboratory and washed thoroughly with running tap water to remove the surface dirt. The leaves were cut into small pieces using sterile scalpel blades and kept in sterile Petri dishes after surface sterilizing with 0.1 percent mercuric chloride for about one minute followed by two changes of sterile water. Further isolation of the fungus was done by performing moist chamber incubation method (Shutleff and Averre, 1997). These surface sterilized pieces were then placed between blotting papers and aseptically inoculated onto Petri dishes containing Potato Dextrose Agar media. The plates were incubated at 25±2°C for 5 to 6 days, and the growth of fungal colonies were recorded every day.

Identification

Fungal colonies were isolated after 5-6 days and pure cultures were transferred to Potato Dextrose Agar slant. The mycelia and spore characters of the fungi were studied under microscope. Fungal isolates were identified on the basis of cultural, morphological and microscopic characteristics viz. mycelium, sporangiophore, spore bearing organ, spore structure etc.

Pathogenicity test of the pathogens

Pathogenicity test was carried out in vitro on the healthy leaves of *Hippophae salicifolia.* The isolated fungal species was cultured on Potato Dextrose Agar (PDA) medium at 25±2°C for 8-10 days in an incubator. Conidial concentration was subsequently adjusted to 1×109 per ml by using haemocytometer to make a spore suspension. Healthy leaves were surface sterilized for 1min with 2% sodium hypochlorite solution (NaOCl). Artificial pricks approximately 2 mm deep on the abaxial surface of leaves were made by sterilized needle. Spore suspension of the test organisms was delivered through a sprayer and lined with moist blotting paper. Leaves sprayed with sterile distilled water served as control. Leaves were incubated at 25±2 °C for 8-10 days.

RESULTS AND DISCUSSION

Extensive and intensive survey revealed that leaf spot disease was prevalent at all the studied populations in Uttarkashi. Microscopic and cultural analysis of the isolated fungi indicates the association of *Alternaria*. Isolation result indicates that *Alternaria tenuissima* was isolated from all the infected leaf samples. The disease symptoms and microscopic characteristics of the pathogens are described as follows.

Symptoms of leaf spot diseases

Leaf spot disease caused by *Alternaria tenuissima* appeared as elongated, circular, water soaked lesions on the abaxial surface of the leaves (Fig. 1). As the disease progressed, the lesions were light brown to gray in colour with an average size of 1 to 5mm in diameter. In later stages, lesions became dry and necrotic. The disease was observed during both the flowering and fruiting periods. In severe infection, the spiny margin of the leaves was twisted inside due to necrosis of the tissues.

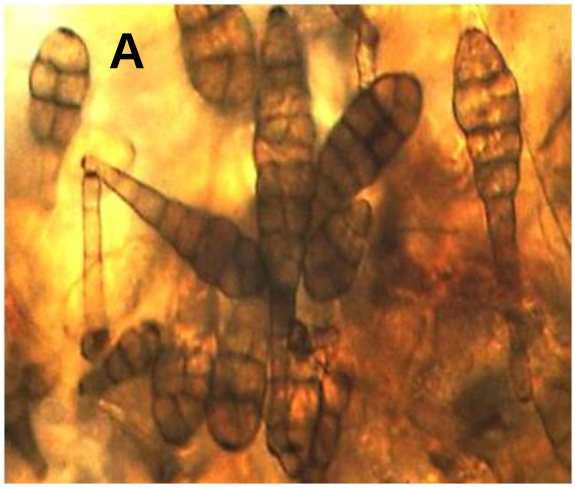
Identification of fungal pathogens

Fungal colonies had a dark olive colour on both sides, with loose, cottony mycelium on the surface of cultures. Simple conidiophores were d”120 ìm long and produced numerous conidia in long chains. Conidia averaged 20.0 × 7.5 ìm and contained two to five transverse septa and the occasional longitudinal septum (Fig. 2 a,b).Based on morphological and cultural characteristics the fungus was identified as *Alternaria tenuissima* (Kunze) Wiltshire (Ref. Culture No. ITCC -1744).

Pathogenicity test

Pathogenicity test was carried out by inoculating, a conidial suspension of 1 x 10^9 conidia ml^{-1} from a single spore culture was spot inoculated onto 10 leaves of a 45 days old plant of sea buckthorn maintained at 25±2°C, 12h/12 h day/night and 90% relative humidity for 72 hours post inoculation. The symptoms of leaf spot disease recorded during the

Fig. 1 : Seabuckthorn plant showing leaf spot



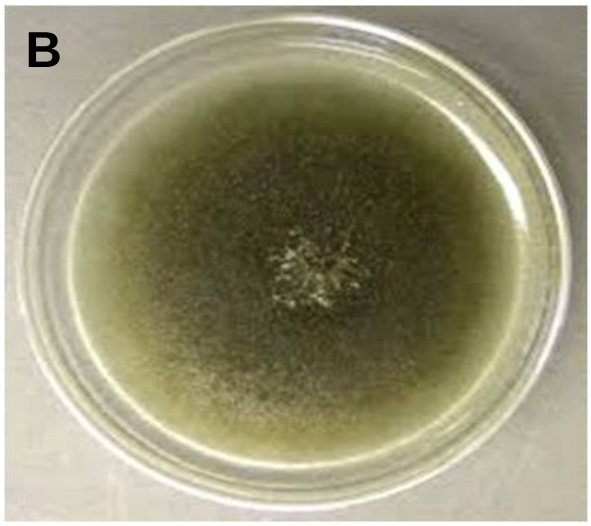


Fig. 2 : A) Macroscopic colony of *A.tenuissima* (X400) B) *A. tenuissima,* 7 day old colony on PDA (X400)

pathogenicity test were almost similar to the natural symptoms. Symptoms of leaf spot infection appeared on fourth day of infestation and by 24 days consisted of pale brown lesions uniformly distributed on leaves. The fungi were re-isolated from the infected leaves and were compared with the original culture of *Alternaria tenuissima*.

Diseases and insects/pests which affect almost every stage/part of the sea buckthorn are the factors affecting its cultivation. At present few pests and diseases of sea buckthorn have been reported; however more are likely to be identified as the number of plantations grow (Kalia *et al.* 2011). The major fungal disease reported on sea buckthorn includes verticillium wilt, fusarium wilt, damping off, brown rot, scab and dried shrink disease in China. The other common pathogenic fungi include the species of *Fusarium, Alternaria*, *Pythiu*m, *Fomes, Monilia, Stigmina hippophae* and *Valsa* (Li, 2003). *Alternaria tenuissima* (Kunze) Wiltshire, a very common hyphomycete on leaf surfaces, as a secondary pathogen or saprotroph. It has been reported causing diseases on 329 hosts (Farr and Rossman, 2015). The fungus is able to infect leaves, branches and fruits. *A. tenuissima* was by far the most frequent species isolated from leaves; and it was highly present on fruits as well. *A.tenuissima* has been identified causing leaf spots on blueberry in Argentina (Wright *et al*. 2008) China; the United States and New Zealand (Farr and Rossman, 2015) It was also reported across Australia on a range of other hosts. *A.tenuissima* species group is one of the most common representatives of the genus *Alternaria* (40). It is important as plant pathogen as well as dangerous for human health, as its growth could result in accumulation of mycotoxins (Stinson *et al*. 1980).

Very few reports are available regarding the pathological aspect of *Hippophae* spp.in India. Incidence of powdery mildew of sea buckthorn was recorded in Himachal Pradesh (Bharat, 2006). Three fungal endophytes *Aspergillus niger*, *Mortierella minutissima* and a sterile mycelium and four species of VAM spores (*Glomus albidum* , *G. fasciculatum,* G. *macrocarpum* and *Gigaspora margariata*) have been isolated from different plant parts and soil samples (Kumar and Sagar, 2007). Root rot caused by *Rhizoctonia solani* is major problem at nursery stage in Uttarakhand (Singh *et al*. 2007). Thus to the best of our knowledge there is no record of occurrence of genus *Alternaria* in as-

sociation with *Hippophae* species in India and *Alternaria tenuissima* is being reported for the first time causing leaf spot disease in *Hippophae salicifolia* D. Don.

The present study provides comprehensive information on pathological aspect of this wonder plant so that proper disease management of this multipurpose species could occur which favours the development and economic potential of sea buckthorn to improve socio economic status of the people residing in its natural habitat. The study will open up new horizon for local farmers and policy makers to develop effective action plan for sustainable use and conservation management of sea buckthorn in cold desert region in particular and Indian Himalayan region in general.

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