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RESEARCH ARTICLE

FIRST REPORT ON OCCURRENCE OF DAMPING OFF DISEASE IN HIPPOPHAE SALICIFOLIA D.DON CAUSED BY FUSARIUM SPECIES FROM UTTARAKHAND, INDIA

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ABSTRACT

Occurrence of damping off disease was observed on *Hippophae salicifolia* D.Don plants at some selected sites in Chamoli, Uttarakhand, India. The typical disease symptoms were observed on the roots and lower stems of plants. Roots turn brown and die after a period of time. On the basis of morphological and microscopic characteristics of the fungus, *Fusarium oxysporum* was found to be associated with the damping off disease. Koch's postulate was applied to confirm the causal organisms of the disease. According to the literature, this is the first report of damping off disease on *Hippophae salicifolia* caused by *Fusarium* from Uttarakhand, and the first report of *Fusarium oxysporum* from India.

INTRODUCTION

The Indian Trans-Himalaya represents a distinct biogeographic zone, characterized by distinct cold arid ecosystem that is spread across Himachal Pradesh, Jammu & 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 sub-species 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, seabuckthorn plant can offer benefits of nutrition, food, medicine, cosmetics etc. to the rural people for their socio-economic development. Seabuckthorn 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 seabuckthorn for ecological, social and economical development, a number of scientific studies have been undertaken in India **(Singh *et al*.,2003; Singh *et al*., 2006; Chauhan *et al*., 2001; Simmons, 1992; Singh *et al*., 2008; Singh *et al*., 2010; Dhyani *et al*., 2007; Rousi, 1971**; **Anonymous, 1997; Butola and Badola, 2008**) and abroad **(Ma, 1989; Rong Sen**, **1990; Rong Sen, 1992; Li, 1999**) but a little work has been done regarding its pathological aspect.

Despite its therapeutic and antimicrobial potential, *seabuckthorn* is susceptible to various fungal diseases. Damping off is an important disease that affects the host when they are in their juvenile's stages of development such as seeds and seedlings. Therefore, it was the objective of this study to identify the causal agent of damping off in *Hippophae salicifolia* D.Don.

MATERIALS AND METHODS

Sample collection and study of symptoms:

Diseased samples were collected from different locations of Chamoli district. The studied populations included Govindghat, Niti and Mana in Chamoli district of Uttarakhand, India. Ten infected root samples were collected randomly from each site, placed in labelled sterile poly bags and taken back to laboratory to study for the presence of damping off causing agent in *Hippophae salicifolia.* The morphology of the symptoms was studied with the help of hand lens and dissecting microscope.

Isolation and purification of pathogen from diseased roots:

Diseased roots of *Hippophae salicifolia* were taken into the laboratory and washed thoroughly with running tap water to remove the surface dirt. The roots 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

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sterilized pieces were then placed between blotting papers and aseptically inoculated onto petridishes 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. and were identified following Barnett and Hunter (1972), Gilmann (1967) and Nagamani *et al.* (2005).

Pathogenicity test of the pathogen

For pathogenicity test, root stabbing method was undertaken in which seedlings having average 10cm in length were taken and planted in pots containing 400 g of sterilized and unsterilized soil/fertilizer mixture (2:1) separately. Pots were kept in triplicates containing sterilized soil mixture and unsterilized soil which serve as control for 15 days. Expression of pathogenicity was observed after inoculation with spore suspension. The spore suspension was prepared. Cultures were harvested by scrapping the surfaces of the colonies with spatula by washing the medium with sterilize distilled water and filtered them through nylon mesh cloth. Spore suspension was adjusted to be 10^6 spores/ml using Haemocytometer and inoculated by plunging a sharp scalpel blade four to six times into the soil surrounding the plant roots and pour the inoculums around it. It should absorb the inoculums and hold it in contact with the roots. Watering may be withheld for 2 days prior to inoculation and resume thereafter.

RESULTS AND DISCUSSION

Extensive and intensive survey revealed that damping off was prevalent at all the studied populations in Chamoli. Microscopic and cultural analysis of the isolated fungi indicates the association of *Fusarium.* Isolation result indicates that *Fusarium oxysporum* was isolated from all the infected root samples. The disease symptoms and microscopic characteristics of the pathogens are described as follows.

Fig 1 Seabuckthorn plant showing damping off

Symptoms of damping off

Post emergence damping off was observed in young seabuckthorn plants. Infection results in lesions at or below soil line (Fig. 1). The seedlings showed wilting, stunting and low vigour. Weak seedlings were found to be more susceptible to attack by one or more fungi when growing conditions are only slightly unfavourable. Roots have some shade of brown or black water soaked areas below the soil line.

Identification of fungal pathogens

Colonies growing rapidly, 4.5 cm in four days on potato dextrose agar. Aerial mycelium white, becoming purple, with discrete orange sporodochia present in some strains; reverse hyaline to dark blue or dark purple. Conidiophores are short, single, lateral monophialides in the aerial mycelium, later arranged in densely branched clusters.

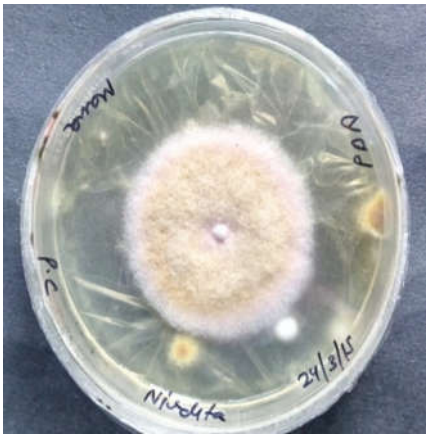


Fig-2(a) *Fusarium oxysporum*, 7-day-old colony on PDA

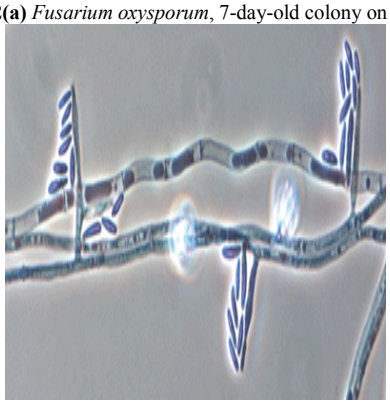
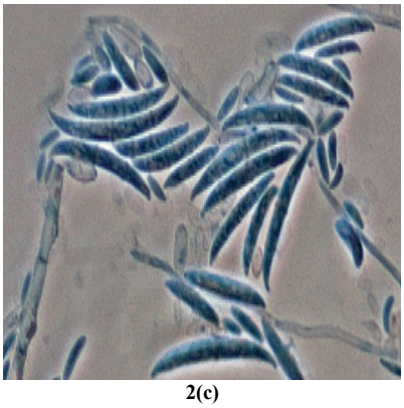


Fig. 2(b)



2(c)

Fusarium oxysporum complex (b) micro conidia on short phialides (c) macro conidia.

Macroconidia are fusiform, slightly curved, pointed at the tip, mostly three septate, basal cells pedicellate, 23-54 x 3-4.5 µm. Microconidia are abundant, never in chains, mostly non-septate, ellipsoidal to cylindrical, straight or often curved, 5-12 x 2.3-3.5 µm. Chlamydospores are terminal or intercalary, hyaline, smooth or rough walled, 5-13 µm. In contrast to *F. solani* complex, the phialides are short and mostly non-septate (Fig 2 b & c). Based on morphological and cultural characteristics the fungus was identified as *Fusarium oxysporum* Schlecht. emend. Synder & Hansen (Ref. Culture No. ITCC-1635).

Pathogenicity test

Pathogenicity test was carried out by inoculating, a conidial suspension of 1x 10^9 conidia ml^{-1} from a single spore culture inoculated onto ten, 45 days old plants of seabuckthorn maintained at 25±2°C, 12h/12h day/night and 90% relative humidity for 72 hours post inoculation. The symptoms of damping off disease recorded during the pathogenicity test were almost similar to the natural symptoms. Symptoms of damping off appeared on tenth day of infestation and by 20 days roots are killed back, causing plants to be stunted and spindly. The fungi were re-isolated from the infected roots and were compared with the original culture of *Fusarium oxysporum*.

DISCUSSION

Occurrence of *Fusarium* spp. is one of the problems, most limiting to growth of seedlings, in nurseries. This pathogen can be transmitted via seeds and damages to the seedlings during pre- and post- emergence stages. Recent work indicates this taxon is actually a genetically heterogeneous polytypic morphospecies **(Donnell & Cigelnik, 1997; Waalwijk *et al*., 1996)** whose strains represent some of the most abundant and widespread microbes of the global soil micro flora **(Gordon & Martyn, 1997).** Diseases and insects/pests affect almost every stage/part of the seabuckthorn. At present few pests and diseases of seabuckthorn 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 seabuckthorn 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, Pythium, Fomes, Monilia, Stigmina hippophae* and *Valsa* (**Li, 2003).** Very few reports are available regarding the pathological aspect of *Hippophae* spp.in India. Incidence of powdery mildew of Seabuckthorn 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 *Fusarium* in association with *Hippophae* species in India and *F. oxysporum* is being reported for the first time causing damping off disease in *Hippophae salicifolia* D. Don.

F.oxysporum strains are ubiquitous soil inhabitants that have the ability to exist as saprophytes. They are also pervasive plant endophytes that can colonize plant roots **(Gordon *et al*., 1997; Katan, 1971)** and may even protect plants or be the basis of disease suppression **(Larkin *et al*., 1993; Lemanceau *et al*., 1993).** Many strains within the *F.oxysporum* complex are pathogenic to plants, especially in agricultural settings. Pathogenic strains of *F.oxysporum* have been studied for more than 100 years. The host range of these fungi is extremely broad, and includes animals, ranging from arthropods **(Teetor *et al*., 1983)** to humans, **(Nelson *et al.,* 1994)** as well as plants which include tomato, tobacco, sweet potatoes, legumes and banana. *F.oxysporum* f.sp. *melonis* attacks muskmelon and cantaloupe. It causes damping off in seedlings and causes chlorosis, stunting and wilting on old plants. Necrotic streaks can appear on the stems.

CONCLUSION

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 seabuckthorn 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 seabuckthorn in cold desert region in particular and Indian Himalayan region in general.

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