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First Report on Phyllospheric Mycoflora of *Hippophae salicifolia* D. Don from India

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ABSTRACT

Hippophae salicifolia D. Don (Sea buckthorn) because of its multifarious benefits is called wonder plant or cold desert gold. Research on medicinal properties and other aspects of seabuckthorn has received much attention in recent past, but there is no information regarding phyllosphere mycoflora of Hippophae salicifolia D.Don in India. During this study 12 fungal genera viz. Alternaria, Aspergillus, Fusarium, Mucor, Trichoderma, Penicillium, Cladosporium, Pythomyces, Cylindrocarpon, Curvularia, Stemphylium and Chrysonilia were recorded for the first time from the leaves of Hippophae salicifolia inhabiting Uttarkashi region of Uttarakhand Himalayas in India.

Key words: *Benefits, cold desert gold, multifarious, Uttarakhand, wonder plant.*

INTRODUCTION

The interaction of microbial communities in phyllosphere influences the safety and survivability of the host plant and the productivity of agricultural crops for human and animal consumption. Phyllosphere is one of the major microbial habitats on the earth, that provides shelter to diverse and complex microbial communities like bacteria, yeast, fungi, actinomycetes, algae, protozoa etc[2,7,11]. Phyllosphere microorganisms influence the growth of their host plant, either negatively as pathogens or positively by increasing the stress tolerance and disease resistance[2,4]. The leaf surface contains different types of stimulatory and inhibitory substance that regulate the microbial colonization on phyllosphere[2,10]. The nature & types of microbial population of the leaf surface particularly the economic crops with leaves have received considerable attention[5,9,13].

Hippophae salicifolia D. Don commonly known as Sea buckthorn is a multipurpose, deciduous, dioecious, thorny and nitrogen fixing shrub-tree growing widely on high altitude regions of Himachal Pradesh, Jammu & Kashmir, Sikkim and Uttarakhand. It is tolerant to extremes of temperature (-43^0 to 45^0 C), resistant to drought conditions and well adapted to the salinity and alkalinity[6,8]. It is supposed to be a store house of nutrients, 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 the socio-economic development. Sea buckthorn leaves are used for antioxidant and other properties.

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The phyllosphere microbes of *Hippophae salicifolia* D. Don may have manifold interactions with the host plant and as there is no report on occurrence of phyllosphere mycoflora of Sea buckthorn from India, the present study is an outcome of survey of naturally growing Sea buckthorn populations from different locations of Uttarkashi district of Uttarakhand, India during fruiting and flowering period by culture dependent methods.

MATERIALS AND METHODS

Sample collection- Leaf samples were collected during flowering and fruiting period in June and October 2014 respectively, 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. Leaf samples were collected in sterile poly bags and taken back to laboratory for isolation of phyllosphere mycoflora.

Isolation- The collected leaf samples were detached aseptically from the branches, cut into small pieces (2-5mm) 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 phyllospheric mycoflora was done by performing moist chamber incubation method[12] and pure cultures of the isolated mycoflora were prepared using various culture media like Potato Dextrose Agar, Czapek's Dox Agar, Malt Extract Agar[1,16] incubating at 28^0 C for 72 hours.

Identification- Fungal colonies were isolated after 3-4 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).

RESULT AND DISCUSSION

Qualitative assessment during flowering period (June, 2014) –Seven genera and fourteen species of fungi were isolated among which one isolate was represented by sterile mycelia. The different fungal genera isolated from phyllosphere during flowering period are *Alternaria, Aspergillus, Fusarium, Mucor, Trichoderma, Penicillium and Cladosporium.*

The genera *Aspergillus and Fusarium* both were represented by three species each (*Aspergillus ustus, A.niger, A.flavus* and *Fusarium oxysporum, F.moniliforme* and *F.sporotrichoides*). The genus *Cladosporium* and Penicillium both were represented by two species each (*Cladosporium sphaerospermum* and *C.oxysporum* and *Penicillium funiculosum* and *Penicillium* sp.). Other three genera *Alternaria, Mucor* and *Trichoderma* were represented by one species each (*Alternaria alternata, Mucor racemosus* and *Trichoderma viride*).

Among the total fungal isolates one genus belonged to Zygomycota (*Mucor*), two genera belonged to Ascomycota (*Aspergillus* and *Penicillium*); four to Deuteromycota (*Alternaria, Fusarium, Cladosporium, and Trichoderma*) and Basidiomycota was represented by sterile mycelium. The maximum representatives are of Deuteromycota (four genera), followed by Ascomycota (two genera) and Zygomycota (one genera) which in return are followed by Basidiomycota (Table 1).

Qualitative assessment during fruiting period (October, 2014) –Twelve genera and twenty species of fungi were isolated from the phyllosphere of seabuckthorn .The genera include *Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Trichoderma, Pythomyces, Cylindrocarpon, Curvularia, Stemphylium* and *Chrysonilia.*

During fruiting period also *Penicillium* was represented by two species (*P. funiculosum* and *Penicillium* sp.). *Aspergillus* was represented by three species (*A.niger, A.ustus* and *A.tetrazonus= Emericella quadrilineata*). The genera *Fusarium, Cladosporium, Alternaria* and Trichoderma each was represented by two species (*Fusarium moniliforme* and *Fusarium sporotrichoides*; *C.sphaerospermum* and *C.oxysporum*; *A.alternata* and *A.humicola; T.album* and *T.viride*).

Other six genera i.e. *Mucor, Pythomyces, Cylindrocarpon, Curvularia, Stemphylium* and *Chrysonilia* were represented by single species each (i.e. *Mucor racemosus, Pythomyces atro-olivaceous,*

Cylindrocarpon heteronemum, *Curvularia tetramera*, *Stemphylium botryosum* and *Chrysonilia sitophila*. Out of these, one genus belong to Zygomycota (*Mucor*), four to Deuteromycota (*Alternaria, Cladosporium, Fusarium, Trichoderma*), seven to Ascomycota (*Aspergillus, Penicillium, Pythomyces, Cylindrocarpon, Curvularia, Stemphylium, Chrysonilia*) and Basidiomycota is represented by two white sterile mycelia. During fruiting period, the number of fungal species isolated from phyllosphere is more than that isolated during flowering period. The new genera reported during fruiting period were *Curvularia, Cylindrocarpon, Pythomyces, Stemphylium,* and *Chrysonilia* (Table 3).

The maximum representative fungal genera belonged to Ascomycota (seven genera), followed by Deuteromycota (four genera) and Zygomycota (one genera). Basidiomycota was also represented by two sterile mycelia (Table 2).

Table 1: Distribution of fungal genera isolated from the phyllosphere of *Hippophae salicifolia* D. Don during flowering period into different sub-divisions of fungi

Sr. No.	Sub-division	Genus
1	Zygomycota	*Mucor*
2	Ascomycota	*Aspergillus, Penicillium*
3	Basidiomycota	Sterile mycelia
4	Deuteromycota	*Alternaria, Fusarium, Cladosporium, Trichoderma*

Table 2: Distribution of fungal genera isolated from the phyllosphere of *Hippophae salicifolia* D. Don during fruiting period

Sr. No.	Sub-division	Genus
1	Zygomycota	*Mucor*
2	Ascomycota	*Aspergillus, Penicillium, Curvularia, Cylindrocarpon, Pythomyces, Stemphylium,* and *Chrysonilia*
3	Basidiomycota	Sterile mycelia
4	Deuteromycota	*Alternaria, Fusarium, Cladosporium, Trichoderma*

Table 3: Comparison of occurrence of different fungal species during flowering and fruiting period

Sr. No.	Name of fungi isolated	Flowering period	Fruiting period
1	*Alternaria alternata*	+	+
2	*Alternaria humicola*	-	+
3	*Aspergillus ustus*	+	+
4	*Aspergillus flavus*	+	-
5	*Aspergillus niger*	+	+
6	*Aspergillus tetrazonus*	-	+
7	*Cladosporium sphaerospermum*	+	+
8	*Cladosporium oxysporum*	+	+
9	*Curvularia tetramera*	-	+
10	*Chrysonilia sitophila*	-	+
11	*Cylindrocarpon heteronemum*	-	+
12	*Fusarium moniliforme*	+	+
13	*Fusarium oxysporum*	+	-
14	*Fusarium sporotrichoides*	+	+
15	*Mucor racemosus*	+	+
16	*Penicillium funiculosum*	+	+
17	*Penicillium sp.*	+	+
18	*Pythomyces atro-olivaceous*	-	+
19	*Stemphylium botryosum*	-	+
20	*Trichoderma album*	-	+
21	*Trichoderma viride*	+	+
22	*Sterile mycelium*	+	+

(+): Indicating presence of the fungus in a particular period, **(-):** Indicating absence of the fungus in a particular period.

The phyllosphere is one of the most prevalent microbial habitats on earth. Recent studies have opened fascinating opportunities for characterizing the spatio-temporal structure of phyllosphere microbial communities in relation with structural, functional, and ecological properties of the host plants, and with physicochemical properties of the environment[3]. The phyllosphere microbes of *Hippophae salicifolia* D. Don may have manifold interactions with the host plant. Similar findings have been reported by earlier workers in most of the angiosperm plants. Regarding phyllosphere fungi of seabuckthorn, it may be mentioned that it is first report of its kind.

Slight fluctuation in the number of fungal species was observed during flowering and fruiting period. These phyllospheric microbes experience fluctuations due to change in environmental factors. Phyllosphere fungi have been isolated with the aim of their exploitation for the overall establishment and welfare of the plant. The phyllospheric mycoflora of seabuckthorn can be exploited as the total extent of lower and upper surfaces of leaves is thought to represent 10^9 km ^{2} that could harbour 10^{26} bacterial cells[14] and is a major potential entrance for phytopathogenic organisms, whose colonization on the plant must not only overcome plant defences, but also confront competition from existing microorganisms. Although their numbers are much lower than those of bacteria, phyllosphere associated fungi are potentially involved in major ecophysiological functions, such as interactions with pathogenic fungi, C/N dynamics or the initial steps of leaf litter degradation[15].

Present study stress the need to undertake in-depth scientific researches on the role of phyllosphere microbiota of Sea buckthorn for the study of mechanism that govern processes at the interface between plants, microorganisms and the atmosphere, either in pristine environments, or in agricultural or anthropogenic environments.

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