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First Comprehensive Report on the Occurrence of Various Fungal Diseases in Seabuckthorn from Uttarakhand, India

Dr. Nivedita Sharma
Department of Allied & Applied Science
University of Patanjali, Uttarakhand, India
Email: maliknivedita8730@gmail.com
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Abstract: Seabuckthorn is a general term given to the deciduous shrub tree *Hippophae* Linn. It is one of the most magical plant resources with higher value of economy and ecology. It is also known as "wonder plant" due to its multifarious benefits. Therefore, seabuckthorn should serve as a measure to safeguard medicinal and nutritional plants, to conserve biodiversity and environment and to generate sustainable income source for local people. Diseases and insects are the major factors affecting the success of seabuckthorn cultivation. Control measures depend on proper identification of diseases and their causal agents. Proper disease diagnosis is therefore vital as without proper identification of the disease and the disease causing agent, disease control measures are waste of time and money and can lead to further plant losses. Despite although there are a few reports available regarding the pathological aspect of *Hippophae* spp. in India, but there is no systematic study on distribution and severity of diseases occurring on *Hippophae salicifolia* D. Don in Uttarakhand. Hence, a systematic study was undertaken on the occurrence of diseases on Seabuckthorn plant which are responsible for negatively affecting the yield and quality of a magical plant of Indian Himalayan Region.

Keywords: diseases, multifarious, pathological, study, systematic.

1. **Introduction:** Genus *Hippophae* Linn. commonly known as Seabuckthorn or Leh Berry is a shrub and/or small tree of the family Elaegnaceae. The name is derived from its habit of growing near the sea, and from possession of many spines or thorns that are reminiscent of some buckthorn species (of the genus *Rhamnus*) (Figure 1.1). In ancient Greece, Seabuckthorn leaves and young branches were added to the fodder for horses which resulted in rapid weight gain and a shiny coat for the horse. This in fact gave the generic name to the plant, in Latin 'Hippo' means horse and 'phaos' means to shine (Rongsen, 1992). Seabuckthorn is considered native to Europe and Asia. It is estimated that the total area of Seabuckthorn in China, Mongolia, and Russia is approximately 810,000 ha of natural stands and 300,000 to 500,000 ha as planted ones (Sun, 1995). Natural Seabuckthorn stands are also widespread in Europe as on river banks and coastal dunes along the Baltic Coast of Finland, Poland and

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Germany (Biswas and Biswas, 1980; Kluczynski, 1989; Rousi, 1971) and on the western coast and along the Gulf of Bothnia in Sweden. In Asia, Seabuckthorn is distributed in the Himalayan regions including India, Nepal, and Bhutan and in the northern parts of Pakistan and Afghanistan (Lu, 1992).

Figure 1.1: *Hippophae salicifolia* D. Don (Seabuckthorn)

In Indian Himalayas, it is believed to cover about 1, 00,000 ha, with world's second largest Seabuckthorn resources (Singh *et al*., 2007). *H. salicifolia* D. Don (Vernacular- Chuk, Tarwa) is the most common and widely distributed species reported to exist in abundance in Indian Himalayan Region (IHR), between 1500-3500 m.a.m.s.l (Hooker, 1894 and Gaur, 1999). In Uttarakhand, it is distributed in the high altitudes of Uttarkashi, Chamoli and and Pithoragarh districts only (Yadav *et al.,* 2006b). Recently Dwivedi *et al.,* (2009) have given the distribution of Seabuckthorn in the different parts of India (Table 1.1). It is one of the best potential species of genus *Hippophae* with regards to high quality fruit, yield and less thorn (Lu *et al.,* 2001). Flowering in June and fruiting ends to October indicating optimum temperature in the high altitudes (Table 1.2).

Table 1.1: Distribution of Seabuckthorn species in India (Dwivedi *et al.*, 2009)

Species (vernacular name)	**Ladakh (Tsermang)**	**Himachal (Chharma)**	**Uttarakhand (Ames, Chuk,Chu)**	**North East (Tare, Taroobo)**
Hippophae rhamnoides	Indus, Nubra, Suru, Changthang valley	Kukumsari, Lakauk, Kaza,Tabo	_	_
Hippophae salicifolia	_	Lahaul	Yamnotri, Kai, Badrinath, gori, Harindun, Buddi, Dharma, Bagnitiyar	Lachen, Lanchug, Dormang

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Hippophae tibetana	Zanskar	Sangrum, Kibbar, Takcha	Gomukh, Niti, Ranimani, Brtal, Nelong, Shinla, Milan	North Sikkim

Table 1.2: General characteristics of Seabuckthorn species found in India (Dwivedi *et al.*, 2009)

Species	Distribution	Altitude (m)	Plant height	Flowering time	Fruit ripening time
Hippophae rhamnoides	India, China, Tibet, Kyrghystan, Kazakistan,Uzbegistan	600-4200	5-6 m	May	September-October
Hippophae salicifolia	India, Tibet, Bhutan, Nepal	2700-3700	3-10 m	June	October
Hippophae tibetana	India, Tibet, China	3000-5200	0.8-1.2 m	May	August-September

Seabuckthorn is a unique and valuable multipurpose plant species having great ecological and social values. It is one of the most magical plant resources used as firewood, fodder and serves as a soil binder in tough and fragile Himalayan terrains. It plays an important role in controlling soil erosion, reclamation of degraded and wastelands, wildlife habitat enhancement etc. The genus is of great ecological significance as roots show excellent soil binding properties. *Frankia* present in its root nodules fixes atmospheric nitrogen making soil more fertile. Natural forest of seabuckthorn can yield 750-1500 kg of berries/ha and seeds also contain high quality oil which has many bioactive substances (Rongsen, 1992). The plant is tolerant to extreme cold, drought conditions, saline and toxic habitats (Rousi, 1971; Li and Schroeder 1999; Personen, 1999). The fruits have a distinctive taste of sour and a unique aroma of pineapple reminiscent. *H. salicifolia* berries are also being used by the local inhabitants in making pickles strengthening the economy of poor in rural areas. Berries are the rich source of carotenoids, minerals, vitamin B, C, E and K. The berries remain on the tree branches all winter until eaten by birds. Scientifically the quality of fruit was recorded as a rich source of vitamins, and used in preparations of various products including local beverages (Gaur, 1999). It has an extensive subterranean rooting system with strong soil binding ability useful for soil stabilization, river bank control and water retention (TISC, 2001). For all these reasons, it is also called a wonderful plant (Lu, 1992). Recently it has attracted considerable attention worldwide mainly for its nutritional, medicinal and environmental values. Pharmacologically, seabuckthorn is considered significant as an anti-inflammatory, anti- microbial and pain relieving (Li, 1999). Various plants have an important historical tradition in healing or are particularly valued for their medicinal and aromatic qualities. Nowadays diseases have becoming a worldwide problem and becoming highly concerning factor. Not only in human beings, animals but also in plants it is becoming a

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problem with various factors like fungi, bacteria, viruses etc. Among these fungi represent the major pathogenic micro-organisms that infect plants, causing numerous diseases. Though seabuckthorn is a multipurpose and vital resource species for mountain rural poors, but at the same time it is also one of the underutilised and unexplored plant species. Vulnerability of the seabuckthorn genotypes to a multitude of biotic stresses has restricted their potential yield. In the mountain areas where there are gregarious natural stands of seabuckthorn but unfortunately at the same time these face heavy health hazards. Diseases and insects or pests which affect almost every part of the seabuckthorn are the major factors affecting its cultivation and yield. At present, only a few pests and diseases of seabuckthorn have so far been reported; however more are likely to be identified as the number of plantations grows viewing its multiple uses (Kalia *et al*., 2011). It is evident from the literature that there are notable contributions on disease incidences on crop plants. But unfortunately, much attention has not so far been paid on the study of diseases related to valuable socio-economic plant of wild as well as cultivated such as seabuckthorn. Therefore, this study was framed with a notion realizing the fact that such studies could yield desirable results in this ignored arena of research.

2. Material and Methods

To achieve the targets of the proposed study district Chamoli of Uttarakhand was selected because of its distinct soil geography and climatology under the influence of which the host plant (*Hippophae salicifolia*) survives and grows. Different sites were chosen for the extensive field survey in order to collect maximum number of diseased plants. Sites were surveyed at regular intervals.

2.1 Survey of sampling sites

An intensive survey on occurrence of pathogenic diseases of *H. salicifolia* was undertaken for one year duration. The repeated visits were made to observe disease incidence at different stage of the plant during flowering (June) and fruiting (Oct) seasons. The diseased plant parts such as leaves, stems, roots and fruits were collected from different sites of Chamoli. The sampling sites taken were Govindghat, Mana, Badrinath, Hanuman Chatti and Rangad where natural stands of *H. salicifolia* were found. Selected sites were visited at regular intervals in order to collect diseased plant specimens.

2.2 Identification and observations of healthy plants

The target plant species i.e. *H. salicifolia* was identified through available literature (Hooker 1894; Gaur, 1999) and further confirmed from the Botanical Survey of India Northern Circle (BSD), Dehradun, obtaining Accession Number. Normal appearance of the specific plant species was determined on the basis of its appearance, growth habit, overall size, shape and colouration and growth rates: leaf shape, size, distribution, leaf drop; root distribution and colouration; bark, stem or trunk texture and colour. The diseased samples were kept

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separately in pre-sterilized air tight polythene bags and brought to the laboratory for the identification and further study of pathogens following standard methods.

2.3 Observation, Identification and Collection of Affected plant

On the basis of variable symptoms on the roots, leaves, stems, flowers or fruits or in the entire plant including:

a) Under or overdevelopment of tissues or organs
b) Necrosis or death of plant parts
c) Disease present on young and old plants
d) The symptoms present on young leaves, old leaves and other plant parts.
e) Occurrence of the like disease in the neighbouring plants of the area.
f) Alteration of normal appearance- Signs were noted in case of biotic causal agents like mycelia, spores and spore producing organs of a fungal agent. Root rot fungi can be diagnosed by cutting the bark at the soil surface by using hand lens and knife. Surfaces of leaves and flowers were observed and studied for rust spores and powdery mildews.
g) Diseased plant materials were collected and placed in paper bags and labelled furnishing following information (s):
    1) Name of the pathogen
    2) Habitat
    3) Locality
    4) Date of collection
    5) Name of collector

2.4 Incubation of diseased plant material

A moist chamber was used for small and flat specimens (Shutleff and Averre, 1997; Waller, Ritchie and Holderness, 1998). Plastic bags or boxes were used for larger specimens. A brief surface swab with 70% isopropanol or 0.1% sodium hypochlorite was used to restrict the growth of saprophytes. Incubation was done at room temperature for 24 to 72 hours to induce the pathogen to yield mycelium, spores and or any fruiting structures for the identification of the pathogen.

2.5 Isolation of causal agents from *H salicifolia*

For the isolation of pathogenic fungal agent, pieces of infected plant tissues were selected from the advancing margins of the lesions, cutting into small pieces using sterile scalpel blades (2-5mm) and kept in sterile petri dishes, then dipped into 0.1 percent mercuric chloride solution for about one minute. Pieces were then transferred to petri plates containing sterile distilled water and washed thoroughly in three changes of sterile water to free them from the chemicals if any. 20 ml of solidified Potato Dextrose Agar/Czapek Dox Agar amended with 60 mg/l chloramphenicole (Tredway *et al*., 2003) to suppress the growth and contamination of bacteria was used to place 2-4 sterilized pieces at different distance in a single PDA plate and incubated at BOD (25±2^{o}C) for 7 days. Hyphal tips developing from the tissue were transferred in PDA slants (Shutleff and Averre, 1997). The pathogen that grew out of this tissue was then isolated in pure cultures (Agrios, 1997). Bacteria were isolated by chopping

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up infected tissue in a small amount of sterile water. This water: bacteria suspension is then streaked onto a bacteriological medium (nutrient agar). Selective media were used for the suspected pathogen.

2.6 Purification of the culture

The organisms isolated from the diseased tissue were purified by the single hyphal tip method. In single hyphal tip method fungal culture was inoculated at the centre of a petri plate containing plain agar (Agar- 15g and Distilled water- 1000 ml). The fungus grows and develops in the form of thin hyphal strands. Petri plates were inverted and examined under the microscope of low power. Hyphal tips were marked with glass marking pencil away from the place of inoculation. A bit agar bearing single hyphal tip was taken out with the help of inoculating needle, placed in PDA slant and incubated at 25^0 C where the hyphal tip grows into a mycelial colony. Pure cultures were maintained on both PDA and CDA medium. Pure cultures were stored in refrigerator for proper identification and further studies.

2.7 Identification/Characterisation of the pathogens

After the successful isolation of the organism, identifications become the next essential step. Microscopic observations were carried out at 400X and 1000X magnifications using compound light microscope. For this purpose, pure colonies of isolates were obtained and then by regular examining of the developed colonies, macroscopic and microscopic specifications were studied and identifications were made. Macroscopic (developing degree of cultures; colour of colonies and changes in colour; colour of colony reverse and change in its colour; colour changes of medium; texture of colony surface; odour; existence of exudates and its situation) and microscopic (habit of hypha, its combination, development of frutification, colour dimension, form of fructification, details of its structure, and all details of spores) specification were studied and identification were made. Further, identification of isolates was also confirmed by consulting various monographs, books and literature available on fungal systematics (Barnett and Hunter, 1972; Gilman, 2001; Ellis, 1971, 1976; Domsch *et al.*, 1980; Raper and Thom 1949; Raper and Fennell, 1965; A.H. Moubasher, 1993). The microphotography of different genera, identified during investigation was carried out using Magnus MIPS-USB (Olympus), DSCW320 (SONY). Identification of pathogens was further confirmed by sending cultures to the National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute (ARI), Maharashtra, Pune, India and Indian Agricultural Research Institute (IARI), New Delhi, India.

2.8 Pathogenicity Testing

Pathogenicity test was performed following the Koch's postulates. *In vivo* evaluation of susceptibility of *Hippophae salicifolia* against isolated pathogens were conducted under greenhouse conditions, at the Department of Plant Pathology, University of Horticulture and Forestry, Ranichauri, Tehri Garhwal. Host plants were grown under controlled conditions and inoculated with the suspected pathogenic organisms following spore suspension spray method, root stabbing method and stem inoculation.

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2.9 Disease assessment and scoring

The field experiment was conducted to record the alteration in morphological characters of healthy and infected plants during the survey on occurrence of pathogenic diseases of *H. salicifolia* at selected sites of Chamoli district in Uttarakhand. A total of 05 sites were surveyed during the study period. Sampling was done in random way by selecting five plots of 100x100m some distance apart from each sampling site. The growth patterns of seabuckthorn plants were observed regularly to record the morphological characteristics of healthy and infected/diseased plants. For this, following parameters were observed:

1. Plant height
2. Leaf length
3. Leaf width
4. Number of leaves
5. Area of spots
6. Colour of spots
7. Number of spots per leaf

2.10 Calculations

A) The disease incidence percentage of each sampling area was calculated by using the formula given by Ginting and Maryono (2009) thereafter, the incidence of disease was classified into following grades on the basis of percentage of leaf area covered by infection (infected spot) (Seif and Hillocks, 1998):

$$\text{Disease Incidence (DI)} = \frac{\text{Number of infected/diseased plants}}{\text{Total number of plants in field}} \times 100$$

B) Frequency of occurrence of isolated fungus was calculated by following formula as described by Abdullah and Al-Mosawi (2010):

$$\text{Frequency of Occurrence} = \frac{\text{Number of plants with fungal genera/species}}{\text{Total number of plants studied}} \times 100$$

3. RESULTS

All the infected parts of seabuckthorn including leaves, stem, root and fruits were collected on the basis of morphological examination as earlier described in the methodology. Various disease symptoms on leaf, stem, root and fruit due to fungal infection have been noted. Based on the symptomology and other indications leaf spot, root rot, fruit rot damping off and fusarium wilt diseases were reported during the study (Plates 3.1-3.5).

3.1 Leaf Spot: The disease was found to be caused by ***Alternaria alternata, Alternaria tenuissima, Curvularia lunata*** and ***Cladosporium sphaerospermum.*** Pathogenicity test was

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done following Koch's postulates using spore suspension spray method under asceptic conditions (Plate 3.1a and 3.1b). Experimental results revealed that leaf spot disease was found to occur at all the sites of district Chamoli during flowering season, whereas, during fruiting season the disease was found to occur at Govindghat, Badrinath, Hanuman Chatti and Rangad (Table 3.1).

$$\text{Disease Incidence (DI)} = \frac{\text{Number of infected/diseased plants}}{\text{Total number of plants in field}} \times 100$$

Plate 3.1a: Leaf spot symptoms on Seabuckthorn

Plate 3.1b: Pathogenicity test using fungal spore suspension

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3.2 Root Rot

Fusarium oxysporum, *Fusarium moniliforme* and *Rhizoctonia solani* were found to cause root rot disease in seabuckthorn. Pathogenicity test was confirmed following Koch's postulates using Root stabbing method. Root rot was prevalent at Govindghat, Mana, Badrinath and Rangad in district Chamoli during flowering season. The prevalence of root rot disease was found to occur at Govindghat and Mana in district Chamoli during fruiting season of the host plant (Table 3.1).

Plate 3.2 a: Root rot symptoms

Plate 3.2 b: Pathogenicity test through Root stabbing method

3.3 Fruit Rot

During the investigation, *Aspergillus niger*, *Aspergillus ustus*, *Penicillium funiculosum and Penicillium chrysogenum* were identified to cause Fruit rot. Pathogenicity test was further confirmed by using spore suspension spray method following Koch's postulates. Fruit rot disease was found to occur at only two sites namely Mana and Badrinath in district Chamoli

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during flowering period. In the fruiting season, fruit rot was only noticed from Badrinath and Rangad sites of Chamoli district (Table 3.1).

Plate 3.3 a: Seabuckthorn berries showing fruit rot

Plate 3.3 b: Seabuckthorn berries showing fruit rot

3.4 Damping Off

Under nursery as well as under natural habitats, *Fusarium oxysporum*, *Fusarium moniliforme*, *Pythium* sp and *Rhizoctonia solani* were the causal organism of deadly soil borne damping off disease in *H. Salicifolia*. Pathogenicity test was performed by using root stabbing method (Plate 3.4a and 3.4b). The disease was prevalent at Govindghat, Mana, Badrinath and Rangad during flowering period. During fruiting season of the host plant damping off disease was found to occur at four sites of district Chamoli i.e. Govindghat, Mana, Hanuman Chatti and Rangad (Table 3.1).

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Plate 3.4 a: Damping off symptoms

Plate 3.4b: Pathogenicity test using root stabbing method

3.5 Fusarium Wilt

During the study period, *Fusarium sporotrichoides* and *Fusarium oxysporum* were found to cause Fusarium wilt in *H. salicifolia*. Pathogenicity test of the suspected pathogen was confirmed by stem inoculation method following Koch's postulates (Plate 3.5a and 3.5b). The disease was found to occur at Govindghat, Badrinath, Hanuman Chatti and Rangad during flowering season of the sampling year. During fruiting season fusarium wilt was not recorded to occur at any of the site (Table 3.1).

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Plate 3.5 a: Fusarium wilt symptoms on Seabuckthorn

Plate 3.5 b: Pathogenicity test under greenhouse conditions

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Table 3.1: Incidences of fungal diseases in district Chamoli during flowering and fruiting season

Sites	Disease Incidence									
	Flowering Season					Fruiting Season				
	LS	LR	FR	DO	FW	LS	LR	FR	DO	FW
Govindghat	+	+	-	+	+	+	+	+	+	+
Mana	+	+	+	+	-	+	+	+	+	+
Badrinath	+	+	+	+	+	+	-	+	-	+
Hanuman Chatti	+	-	-	-	+	+	-	-	+	-
Rangad	+	-	-	+	+	+	-	-	+	-

Indications: LS- Leaf spot; RR- Root Rot; FR- Fruit Rot; DO- Damping off; FW- Fusarium wilt; absent; += present

3.6 Disease Incidence percentages during flowering season

Disease incidence (%) was calculated during flowering period of 2014 from the sampling sites of district Chamoli. Disease incidence was 35.29 in Govindghat during the flowering season of first sampling year whereas in Mana it was 40.74 during the above sampling year. Badrinath shows disease incidence percentage of 35.29 in year 2014 (Table 3.2; Figure 3.1).

Table 3.2: Disease incidence (%) Flowering season

District	Sites	Total Number of Plants	Diseased Plants	Grade	Disease Incidence (%)
2014					
Chamoli	Govindghat	34	12	2	35.29
	Mana	27	11	2	40.74
	Badrinath	37	10	1	35.29
	Hanuman Chatti	35	12	1	27.02
	Rangad	22	10	2	45.45

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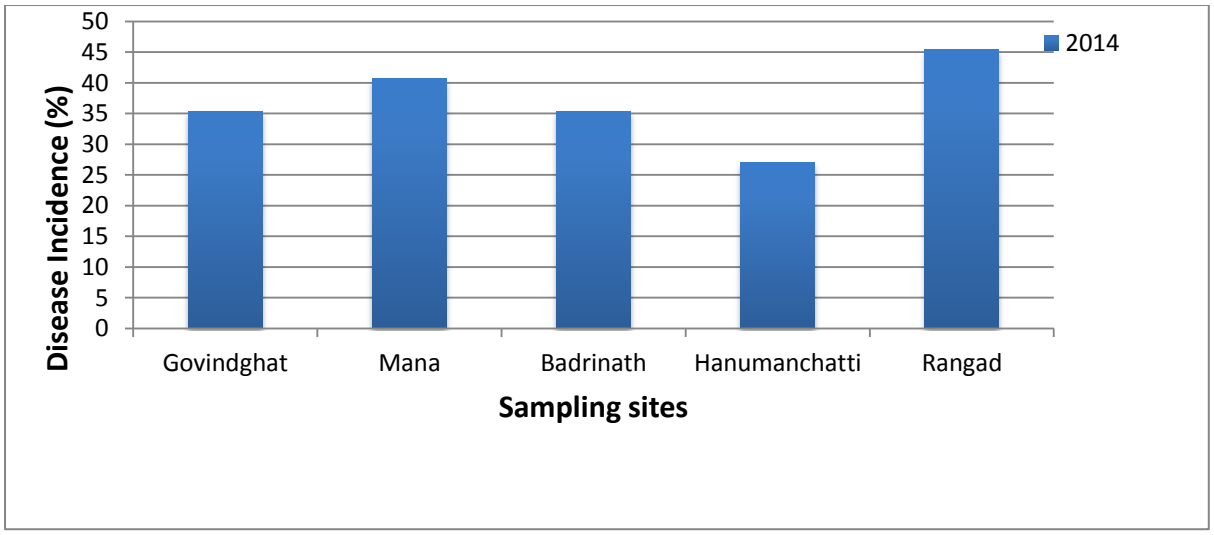

Figure 3.1: Disease incidence (%) during (Flowering season)

3.7 Disease Incidence percentages during fruiting season

Disease incidence was 46.66 in Govindghat while it was 40.74 in Mana during fruiting period of first sampling year. Badrinath showed disease incidence percentage of 45.71 and Hanuman chatti showed 39.47 during the respective year. In Rangad it was 60.00 during the fruiting period of sampling year (Table 3.3; Figure 3.2).

Table 3.3: Disease incidence (%) Fruiting season

District	Sites	Total Number of Plants	Diseased plants	Grade	Disease Incidence (%)
Chamoli	2014				
	Govindghat	30	14	2	46.66
	Mana	27	11	2	40.74
	Badrinath	35	16	1	45.71
	HanumanChatti	38	15	1	39.47
	Rangad	30	18	2	60.00

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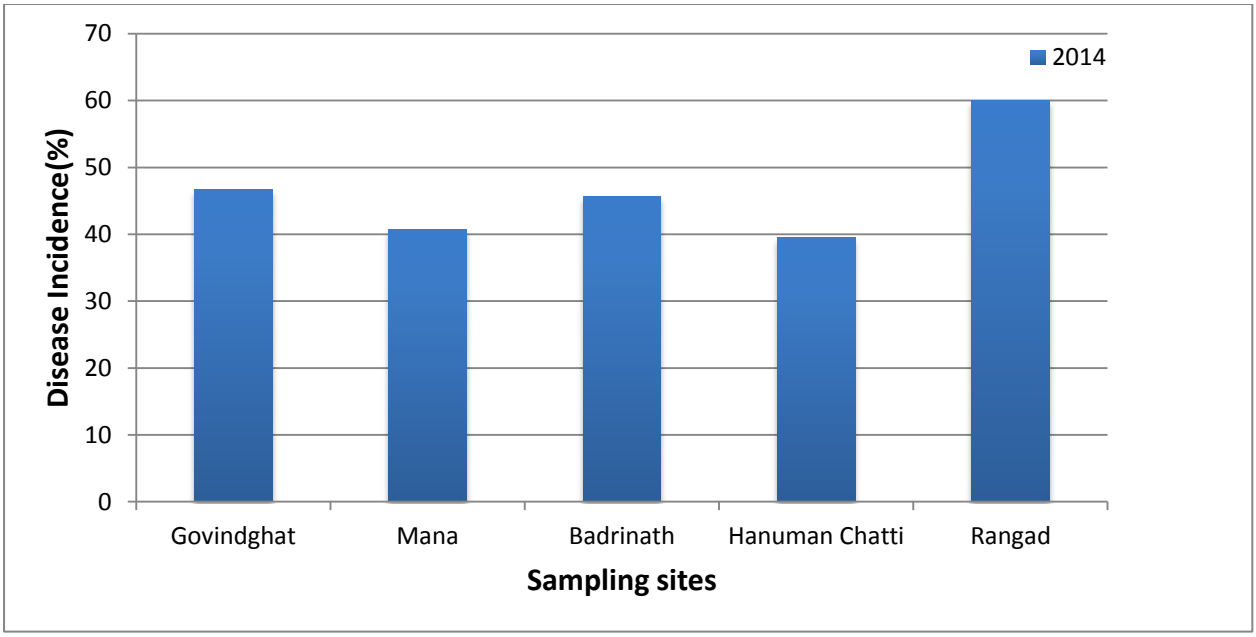

Figure 3.2: Disease incidence (%) during (Fruiting season)

3.8 Isolation and identification of fungi

Diseased parts of *Hippophae salicifolia* i.e leaves, stem, roots and fruits exhibiting disease were analyzed for their fungal infection. Isolation results revealed that a total twenty five fungal species belonging to fifteen different genera were found to be associated with *Hippophae salicifolia* as the causal organisms of various diseases. A total of thirteen species were isolated from all the five sampling sites during the flowering period and nineteen during the fruiting period.

Table 3.4: Frequency (%) of fungal species isolated from *H. salicifolia* during 2014 (Flowering Season)

Fungi Isolated	(Frequency %)				
	Chamoli (2014)				
	Govindghat	Mana	Badrinath	Hanuman chatti	Rangad
Aspergillus ustus	30	32.5	26.25	33.33	-
Aspergillus niger	-	33	28.5	-	-
Aspergillus flavus	-	22	-	16.66	-
Fusarium oxysporum	15	10	17	-	-
Fusarium moniliforme	18	15	27.27	-	28.25
Fusarium sporotrichoides	20	-	17.5	15.25	11.4

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Cladosporium sphaerospermum	17	25	14	22	13
Cladosporium oxysporum	16	17.5	-	-	-
Penicillium funiculosum	12	20.22	18.18	-	-
Penicillium chrysogenum	9	16	14.25	-	17.3
Alternaria alternate	18	15	10	11	8.4
Mucor racemosus	8	6.25	5.25	-	-
Trichoderma viride	**3**	-	-	-	-
Total fungal species	**11**	**11**	**10**	**5**	**5**

Table 3.5: Frequency (%) of fungal species isolated from *H. salicifolia* during 2014 (Fruiting Season)

Fungi Isolated	**(Frequency %)**				
	Chamoli (2014)				
	Govindghat	**Mana**	**Badrinath**	**Hanuman Chatti**	**Rangad**
Penicillium funiculosum	30	32.50	-	33.33	-
Penicillium chrysogenum	27.21	24.35	26.45	-	17.32
Aspergillus niger	28	25.35	23.36	16.66	18.36
Aspergillus ustus	15	10	-	-	-
Aspergillus tetrazonus = Emericella quadrilineata	-	22.57	27.27	-	19.32
Fusarium moniliforme	20	17.50	11.76	-	15.22
Fusarium sporotrichoides	-	11.22	-	9.58	-
Cladosporium sphaerospermum	15	7.50	16	-	14.23
Cladosporium oxysporum	14.24	16.38	18.18	-	13.22
Alternaria alternate	18	17	22	14	17.56
Alternaria tenuissima	14	9	-	16.24	18.52
Trichoderma album	2.50	-	-	4.63	-
Trichoderma viride	-	2.36	-	-	-
Mucor racemosus	-	2.56	-	3.23	-

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Phythomyces atro-olivaceous	10	-	16.66	-	11.53
Cylindrocarpon heteronemum	16.66	15.32	-	14.34	-
Curvularia lunata	20	18	-	16	12.56
Stemphylium botryosum	6.50	-	3.56	-	6.52
Chrysonilia sitophila	-	8.5	-	4.25	-
Total fungal species	**14**	**16**	**9**	**10**	**11**

3.9 Disease Diagnosis: During morphological examination, all parts of Seabuckthorn plant i.e. leaves, stem, root and fruits were found to be infected. Various disease symptoms on leaf, stem, root and fruit due to fungal infection were carefully noted. Interestingly, no bacterial disease was found during the study period. On the basis of symptomology, leaf spot, root rot, fruit rot, damping off and Fusarium wilt diseases were of common occurrence during the study period. Leaf spot disease was found to be caused by *Alternaria alternata*, *Alternaria tenuissima*, *Curvularia lunata* and *Cladosporium sphaerospermum*. *Fusarium oxysporum*, *Fusarium moniliforme* and *Rhizoctonia solani* were the causal organism of Root rot. *Aspergillus niger*, *Aspergillus ustus*, *Penicillium funiculosum* and *Penicillium chrysogenum* were associated with Fruit rot. *Fusarium oxysporum*, *Fusarium moniliforme*, *Pythium* sp., and *Rhizoctonia solani* are responsible for deadly soil borne Damping off disease of Seabuckthorn. Fusarium wilt was found to be caused by *Fusarium sporotrichoides* and *Fusarium oxysporum*. Following table summarizes the diseases and pathogens identified and isolated during the study (Table 3.6).

Table 3.6: Fungal diseases and their causal agents reported during the investigation

1.	Leaves- Leaf Spot	*Alternaria alternata, Alternaria tenuissima, Curvularia lunata Cladosporium sphaerospermum*
2.	Root - Root Rot	*Fusarium oxysporum., Fusarium moniliforme, Rhizoctonia solani*
3.	Root - Damping off	*Fusarium oxysporum, Fusarium moniliforme, Pythium* sp., *Rhizoctonia solani.*
4.	Stem – Fusarium Wilt	*Fusarium sporotrichoides, Fusarium oxysporum*
5.	Fruit – Fruit Rot	*Aspergillus niger, Aspergillus ustus, Penicillium chrysogenum, Penicillium funiculosum*

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4. CONCLUSION

In Uttarakhand, the natural habitats of Seabuckthorn are under threat partly due to some reasons and largely to anthropogenic reasons. Diseases are of natural origin and like other plants, Seabuckthorn also faces many losses to various pathogens and fungi are of prime importance causing various diseases of this magical plant of fragile ecosystems. Therefore, in the present study efforts were made to explore the pathogenic diseases of *Hippophae salicifolia* D. Don in two provenances of Garhwal Himalayas and to search for their bio-control agents. During the study, leaf spot, root rot, fruit rot, damping off and fusarium wilt have been reported for the first time in Uttarakhand. Though these are common diseases of other plants too, but nonetheless, such diseases associated with such a valuable plant of harsh climate conditions of multiple use open new era of research in this ignored field.Among the diseases, root diseases play an important part in reducing the yield of medicinal plants because the health of the root system is the most important determining factor in the total health of the plant as plants grow at expense of the root system (Tattar, 1989). Harsh and Gupta (1993) have estimated nearly 70% mortality of seedlings in nurseries due to root diseases in Central India. Diseases like downy and powdery mildews root, damping off, seedling rots, leaf blights, root rots, leaf spot, leaf rust and die back have been reported in different species of medicinal plants. Occurrence of Damping off disease was observed on *Hippophae salicifolia* 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. The disease is a major cause of mortality in Seabuckthorn seedings in this part of Himalayan region. Although Damping off disease in Seabuckthorn was found to be caused by a number of fungal agents like *Fusarium oxysporum*, *Fusarium moniliforme*, *Pythium* sp. and *Rhizoctonia solani* but *Fusarium oxysporum* was identified as a main cause of this disease. Koch's postulate was applied to confirm the causal organisms of the disease. Damping off disease caused by *F.oxysporum* in Seabuckthorn is not a maiden report from Uttarakhand but not otherwise mentioned from India also. The disease is responsible for a large number of seedling mortality in the region. Root pathogens like Damping off are said to be major limiting factors for plant growth and yield. They reduce the ability of roots to absorb water and nutrients by penetrating tissues of roots. 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 that this taxon is actually a genetically heterogeneous polytypic morphospecies (Donnell and Cigelnik, 1997; Waalwijk *et al*., 1996) whose strains represent some of the most abundant and widespread microbes of the global soil micro flora (Gordon and Martyn, 1997**).** Among the twelve *Fusarium* species identified in Russia, *F. oxysporum* var orthoceras was the most widespread, and *F. sporotrichoides* was the most aggressive (Orellana, 1971). Seabuckthorn wilt caused by *Fusarium sporotrichoides* is being reported for the first time from Chamoli region of Uttarakhand Himalayas in India in this study. *F. sporotrichoides* and *F. acuminatum* were reported causing pink discoloration of the sunflower pith in addition to *F. oxysporum* in northern Great Plains (Leslie and Summerell, 2006). *Fusarium sporotrichoides* has been reported to cause foliar spots on Forage Corn in Chile. Certain graminaceous plants such as

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Zea mays and *Triticum aestivum* serve as host for *Fusarium sporotrichoides* (Tomoya *et al.*, 2012). *Fusarium sporotrichoides* is a frequent pathogen in corn silage (Baath *et al*,. 1990) and cereal crops (Leslie and Summerell, 2006; Vargo and Baumer, 1986). Leaf spot disease was observed on *Hippophae salicifolia* 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. Only on a few sites like Govindghat of Chamoli district this disease was also found to be caused by *Curvularia lunata* during flowering and fruiting season but as a rare. Available literature unreveal that there is no record of occurrence of genus *Alternaria* in association with *Hippophae* species in India and *Alternaria tenuissima* is being reported for the first time causing leaf spot disease in *Hippophae salicifolia*.

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