Effect of Entomological and pathological agents on the vegetative propagation, growth and yield of Natural and Nursery population of *Hippophae salicifolia* D.Don.

THESIS

SUBMITTED TO THE

V. C. S. G. Uttarakhand University of Horticulture and Forestry, Bharsar, Uttarakhand, India

College of Forestry, Ranichauri, Tehri-Garhwal, Uttarakhand

Submitted by

Charan G S
B.Sc. (Forestry)

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science in Forestry
(Forest Products and Utilization)

July, 2017

DEPARTMENT OF FORESTRY

College of Forestry

(VCSG Uttarakhand University of Horticulture and Forestry)

Ranichauri, Tehri Garhwal-249 199, Uttarakhand

Tele phone: 01376-252644, (M) 8476004114

Email: khandurivp@yahoo.com

Dr. V. P. Khanduri

Head, Dept. of Forestry

CERTIFICATE

This is to certify that the thesis entitled **"Effect of Entomological and pathological agents on the vegetative propagation, growth and yield of Natural and Nursery population of *Hippophae salicifolia* D.Don"** submitted in partial fulfilment of the requirements for the degree of **Master of Science in Forestry** with major in **Forest products and Utilization** of the College of Post-Graduate Studies, VCSG Uttarakhand University of Horticulture & Forestry, Bharsar, is a record of ***bonafide*** research carried out by **Mr. Charan G S, Id. No. UUHF/15357,** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

(Dr. V. P. Khanduri)

Chairman

Advisory Committee

DEPARTMENT OF FORESTRY

College of Forestry

(VCSG Uttarakhand University of Horticulture and Forestry)

Ranichauri, Tehri Garhwal-249 199, Uttarakhand

Tele phone: 01376-252644, (M) +91 8476004114

Email: khandurivp@yahoo.com

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Mr. Charan G S, Id. No. UUHF/15357,** a candidate for the degree of **Master of Science in Forestry** with major in **Forest products and Utilization**, agree that the thesis entitled **"Effect of Entomological and pathological agents on the vegetative propagation, growth and yield of Natural and Nursery population of *Hippophae salicifolia* D.Don"** may be submitted in partial fulfilment of the requirements for the degree.

(Dr. R.S Bali)	**(Dr. Laxmi Rawat)**	**(Dr. C.S Dhanai)**
Co-Advisor	Member	Member

(Dr. V. P. Khanduri)

Advisor and Chairman

Advisory Committee

DEDICATED TO

MY DEAR PARENTS

AND

'MOTHER NATURE'

Acknowledgement

"During one of my visit to work site Yamunotri, I was alone, isolated from the rest of the world, in the Hippophae jungle. That is where I consciously encountered the majestic mountains of Yamunotri. Just staring at her, I realized that I am just a speck of dust that is too small to grasp the vastness of nature. It was the greatest realization in my life. Deep down my heart, it broke my supremely held ego into temporary pieces of life. From then I have found a meaning to my life. It is the best result that I am taking home with me from this research".

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Ranichauri Campus **(Charan G S)**

July, 2017 **Author**

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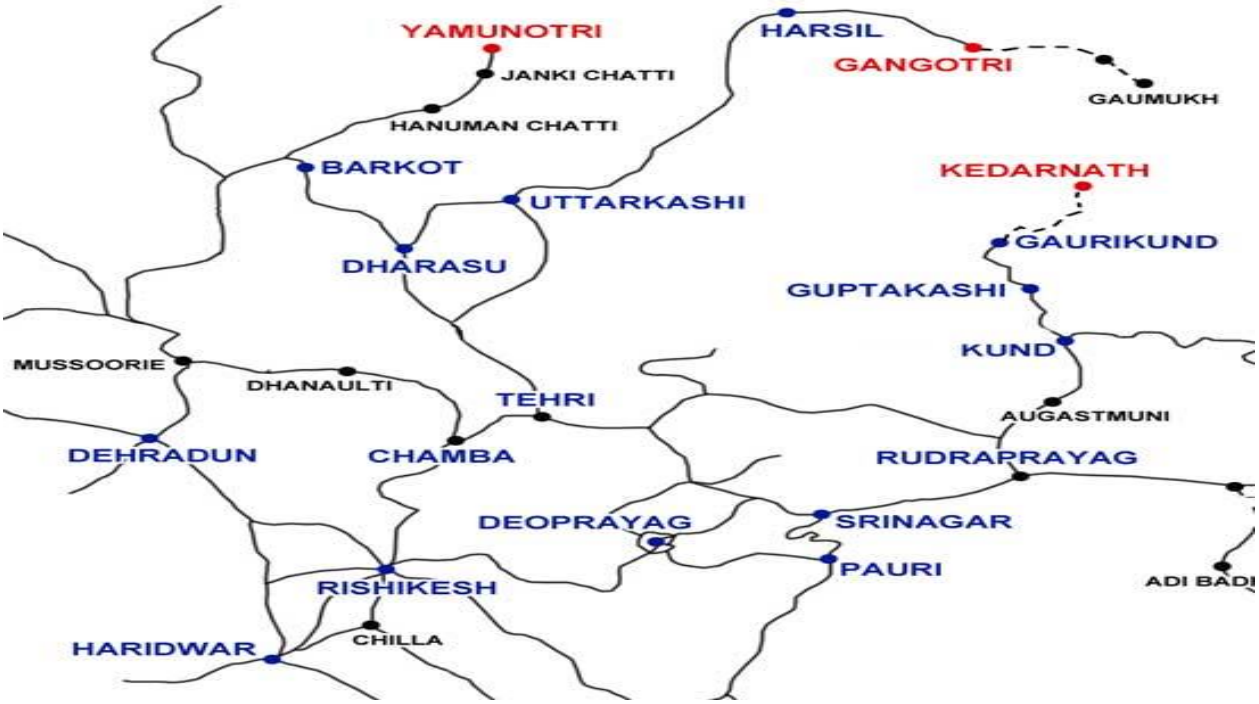
ABBREVATIONS USED

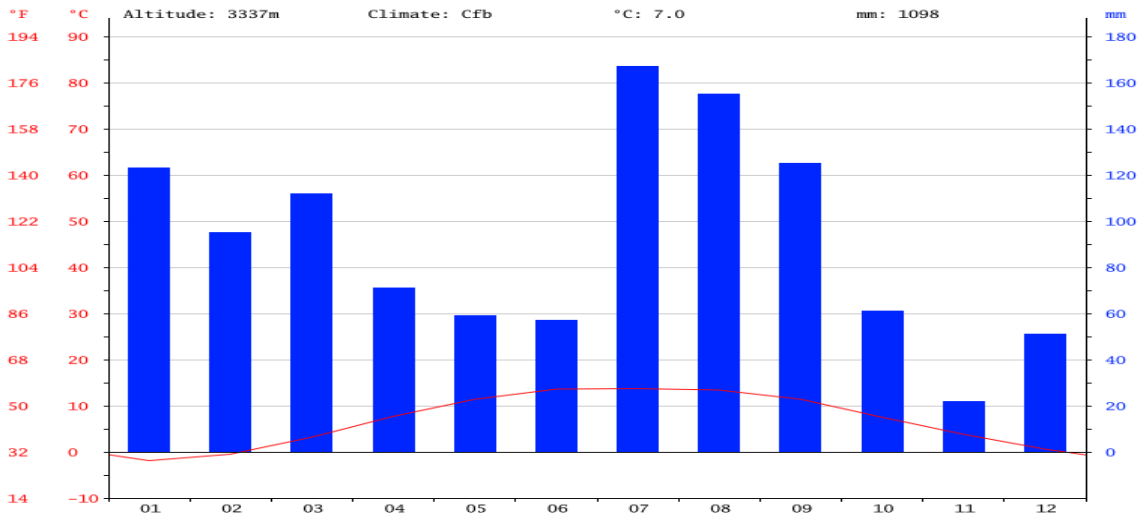
Sl. No.	Particulars	Full Form
1.	Cal	Calorie
2.	cm	Centimetre
3.	gm	Gram
4.	kg	Kilogram
5.	km	Kilometre
6.	m	Metre
7.	mm	Millimetre
8.	NGO	Non-Governmental Organization
9.	ppm	Parts per million
10.	Spp	Species
11.	PGR	Plant Growth Regulators
12.	ml	Millilitre
13.	ASML	Above Mean Sea Level
14.	ft	Feet
15.	IBA	Indole Butryic Acid
16.	@	At the rate
17.	%	Percentage
18.	^{0} C	Centigrade
19.	SBT	Seabuckthorn

CHAPTER-1

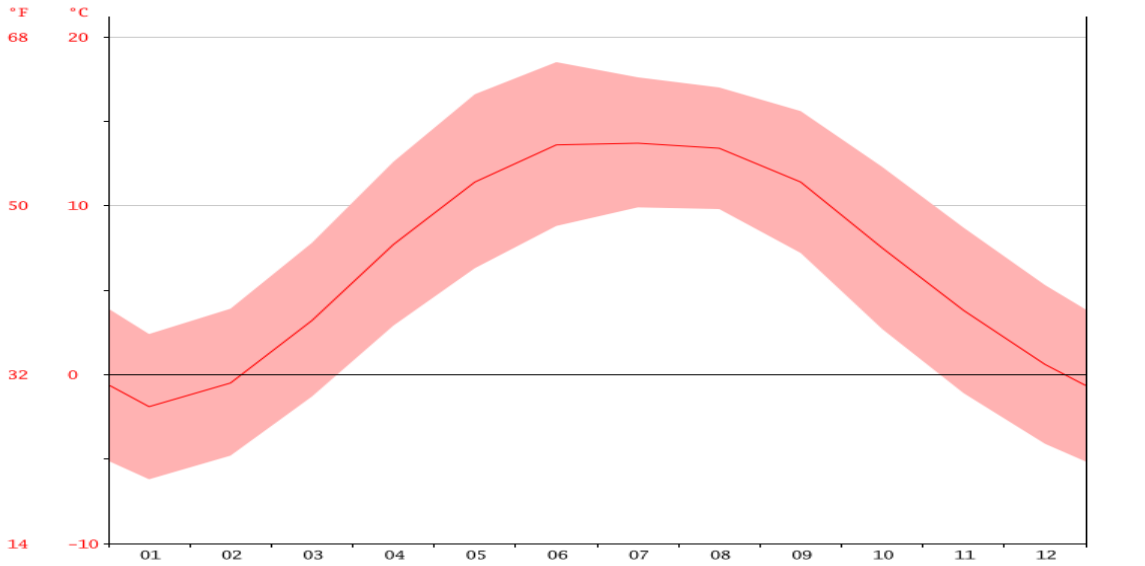
INTRODUCTION


भारत
INDIA
Uttarkashi
Dehradun
Rudraprayag
Tehri
Garhwal
Chamoli
Haridwar
Garhwal
Bageshwar
Almora
Pithoragarh
Nainital
Champawat
उत्तराखण्ड
UTTARAKHAND
Udham Singh
Nagar


YAMUNOTRI
JANKI CHATTI
HANUMAN CHATTI
HARSIL
GANGOTRI
GAUMUKH
BARKOT
UTTARKASHI
KEDARNATH
GAURIKUND
DHARASU
GUPTAKASHI
KUND
MUSSOORIE
DHANAULTI
TEHRI
AUGASTMUNI
DEHRADUN
CHAMBA
RUDRAPRAYAG
DEOPRAYAG
SRINAGAR
PAURI
RISHIKESH
CHILLA
HARIDWAR

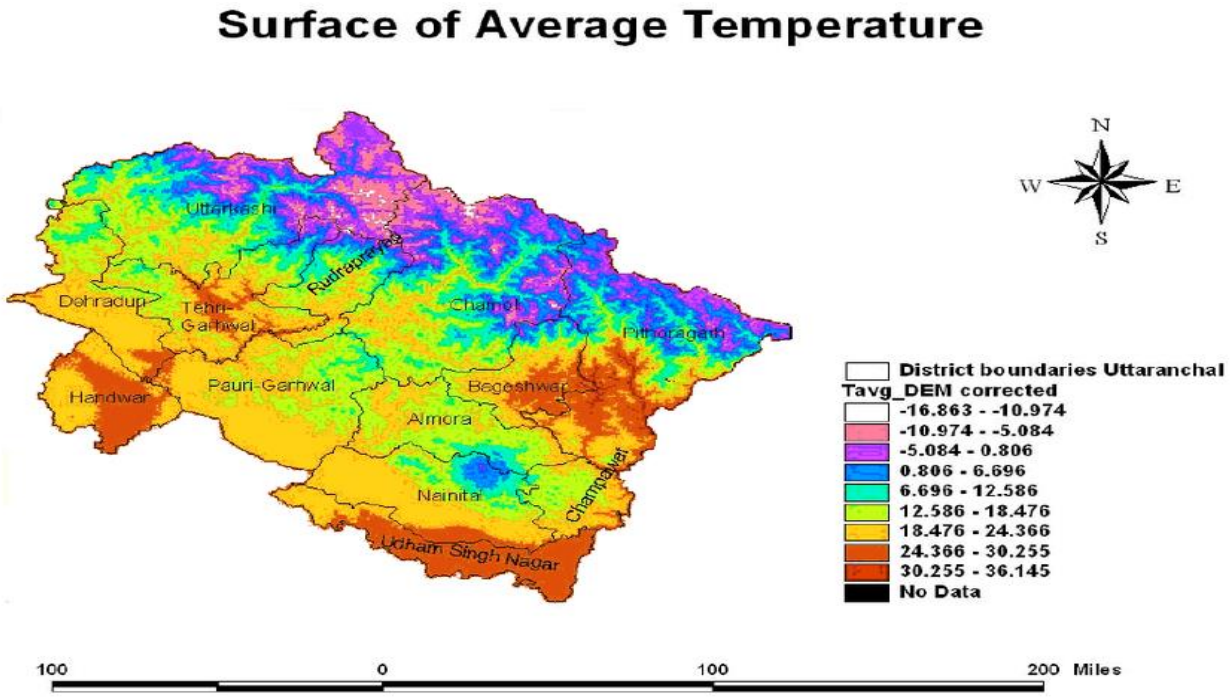


Climate graph of Yamunothri: The driest month is November. There is 22 mm of precipitation in November. Most of the precipitation here falls in July, averaging 167 mm.



Temperature graph of Yamunothri: With an average of 13.7^0 C, July is the warmest month. January is the coldest month, with temperature averaging -1.9^0 C.

(Climatedata.org)



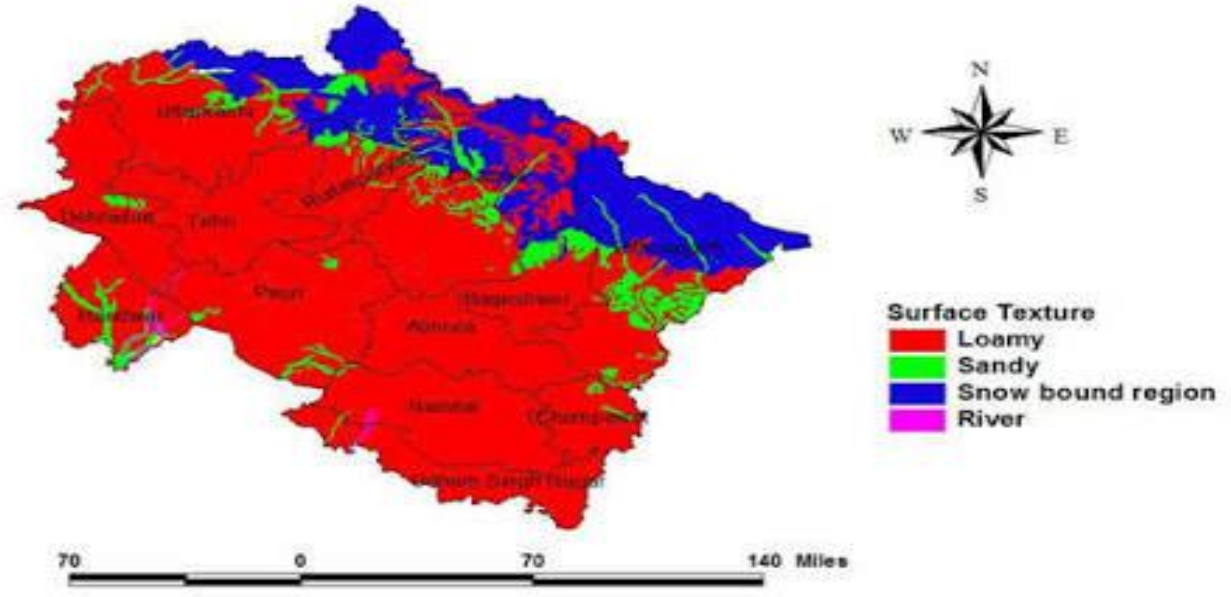


Fig. 2: Soil Surface texture of Uttarakhand

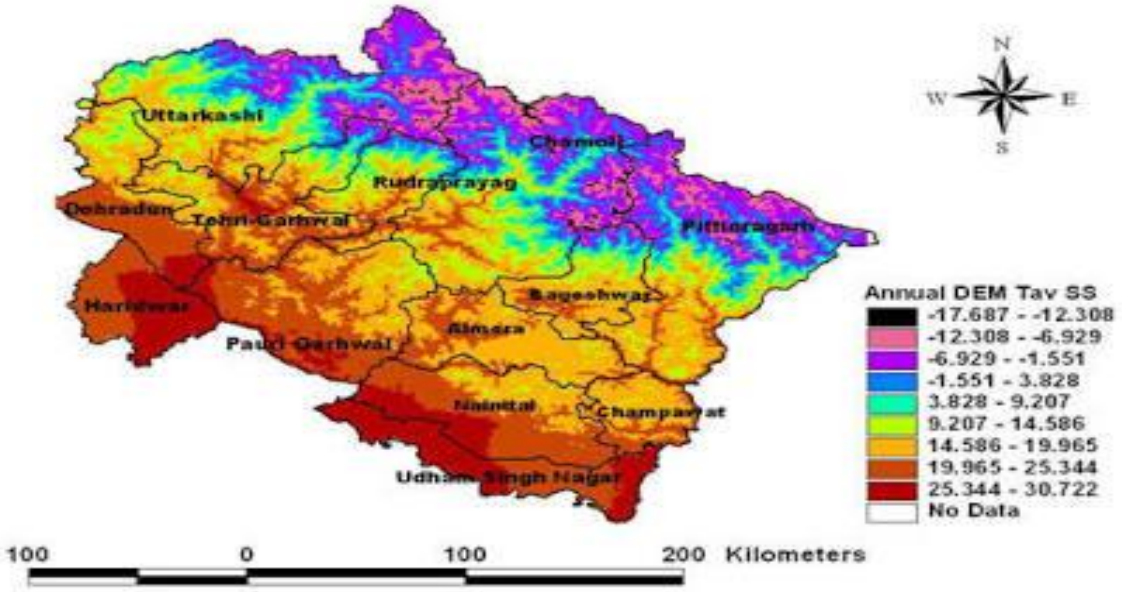
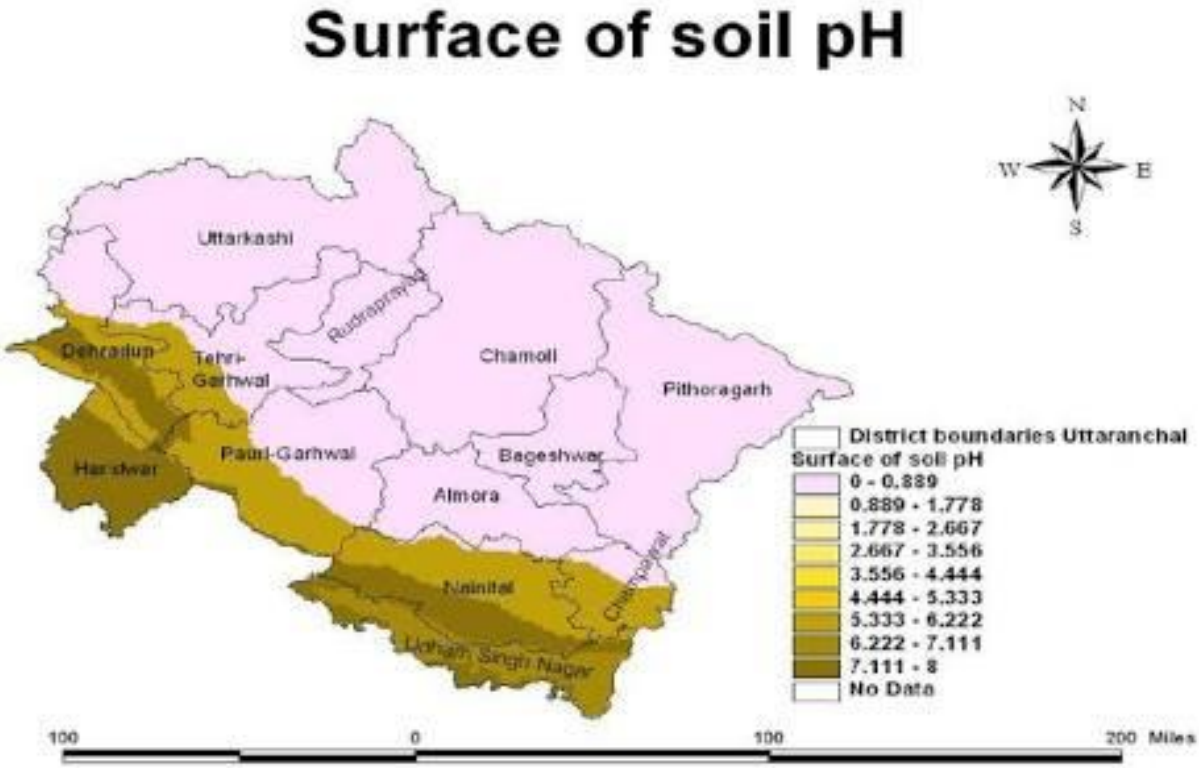


Fig. 3: Thematic map of average temperature over Uttarakhand



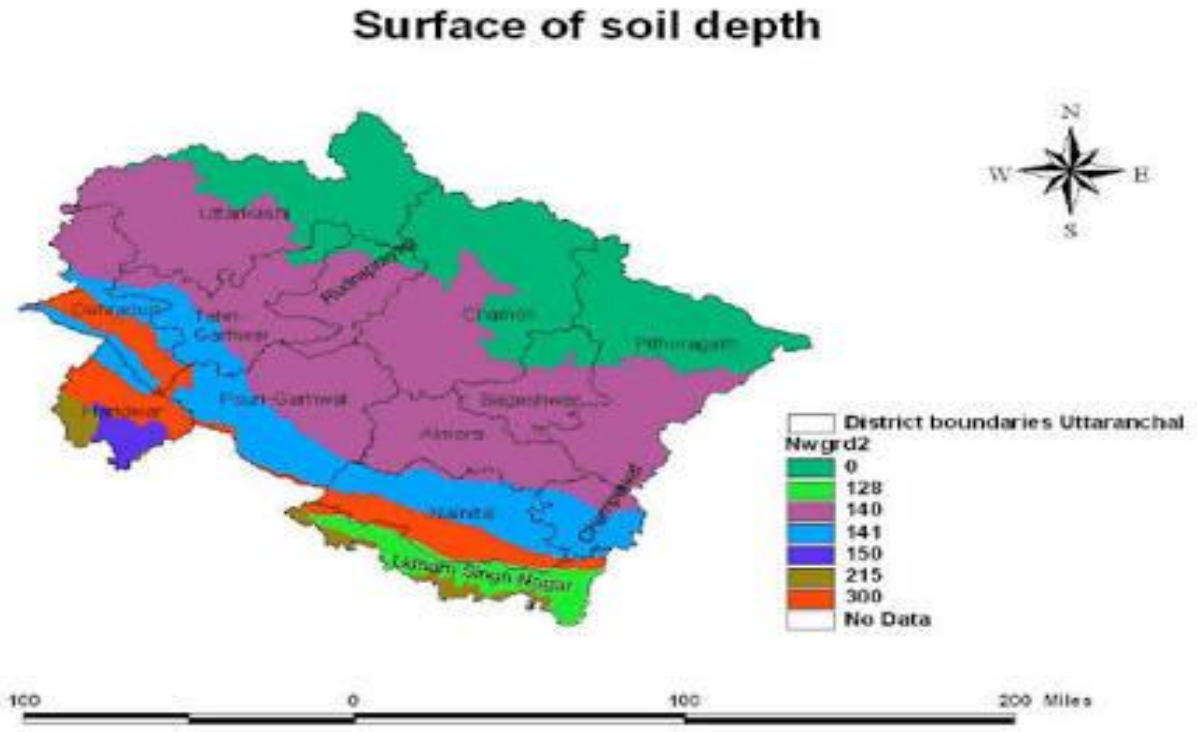


Photo: A view of Yamunothri glacier

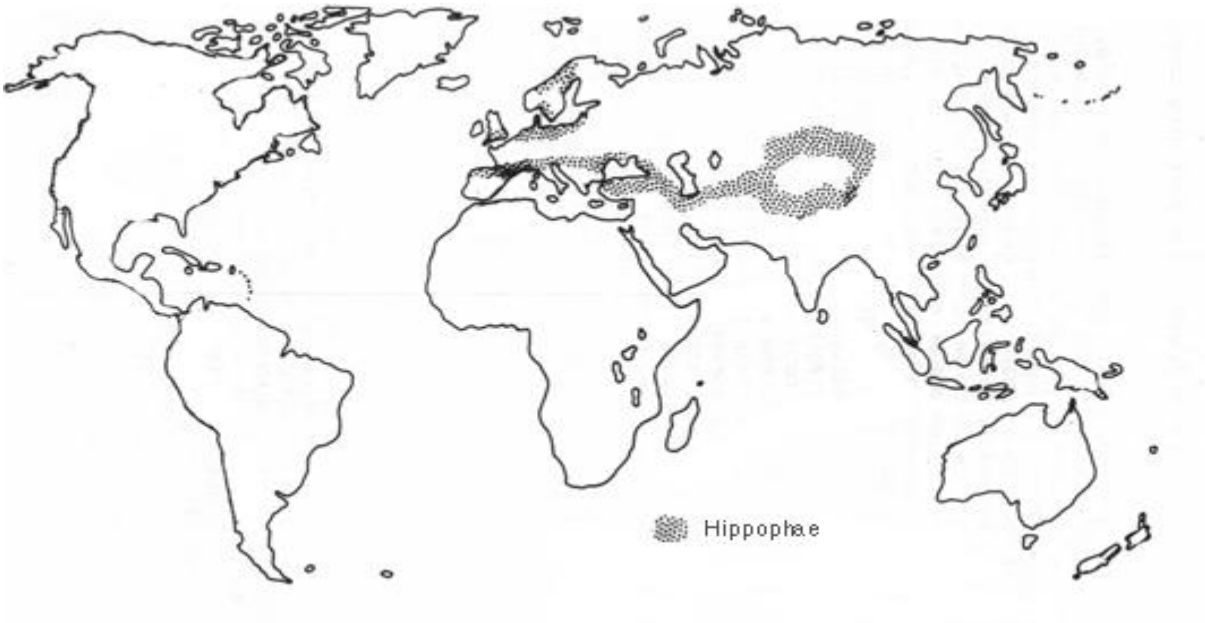
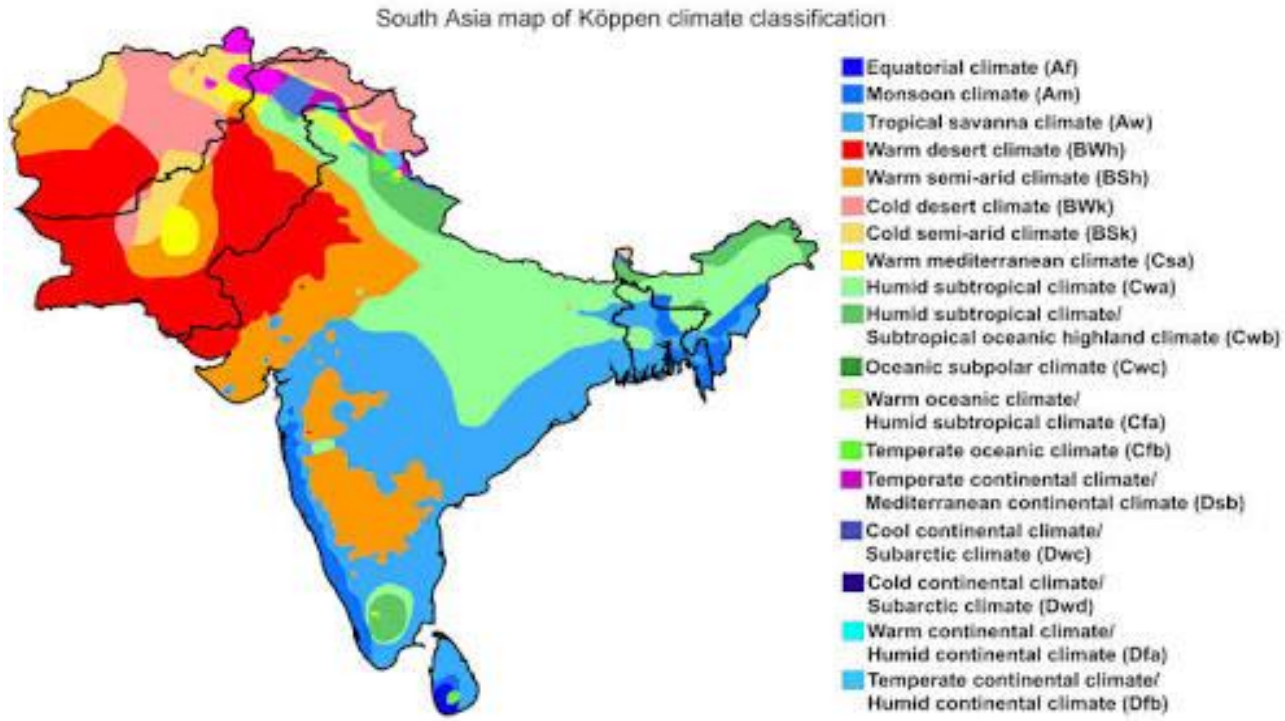


Photo: Distribution of *Hippophae* in the world



India and South Asia: Koppen climate map with legend

CLIMATE TABLE / HISTORICAL WEATHER DATA OF YAMUNOTHRI

MONTH	Avg. Temperature (°C)	Avg. Temperature (°F)	Precipitation / Rainfall (mm)
January	-1.9	28.6	123
February	-0.5	31.1	95
March	3.2	37.8	112
April	7.7	45.9	71
May	11.4	52.5	59
June	13.6	56.5	57
July	13.7	56.7	167
August	13.4	56.1	155
September	11.4	52.5	125
October	7.5	45.5	61
November	3.8	38.8	22
December	0.6	33.1	51

The precipitation varies 145 mm between the driest month and the wettest month. Throughout the year, temperatures vary by 15.6 °C.

CHAPTER 1 – INTRODUCTION

1.1 General Description

Seabuckthorn is a general term used for medicinal, non-leguminous, nitrogen fixing, deciduous and dioecious, shrub-tree, Hippophae Linn., which belongs to the family Elaeagnaceae (Naithani and Bist, 2009).

1.2 General Distribution

In the world, it is distributed in the Hindu Kush Himalayas, adjoining areas of China, parts of Europe and the USSR as well as in the Scandinavian region. It has been reported from about 30 countries of Asia, Europe, South and North America (Dwivedi *et al*., 2009). In these countries seabuckthorn has been distributed along the coastal areas of seas (Singh & Dogra, 1996). Taxonomically, Hippophae is a very complex genus and comprises of 6 species and 7 sub-species, distributed throughout Asian mountains and Europe in the world (Chaurasia *et al.,* 2009). In India, it is mainly distributed in higher elevations of Indian Himalayas, from Ladakh (J&K), to Lahaul-Spiti (H.P), Kumaon and Garhwal in Uttarakhand, Sikkim and Arunachal Pradesh (Chauhan, 1995). Area wise, in India distribution of Hippophae in some states is as follows: Leh-11,500 ha, Uttarakhand - 3750 ha) (Stobdan *et al*., 2008; Yadav *et al*., 2009).

1.3 General Habitat

Seabuckthorn generally grows on riverbanks. It is widely distributed throughout the temperate zones up to an elevation of 5,200 m. It can resist low temperature up to 43◦C and can withstand temperature as high as 40◦C. Under natural conditions, it grows on slopes, well drained soil with silt, river banks, lakes, seashores and wastelands. It is well adapted to dry conditions and it has root nodules due to nitrogen fixing bacteria, Frankia (Chaurasia *et al*., 2009). Seabuckthorn shows a great range of morphological variations in growth pattern, bark, leaves, flower buds, flowers, pollen morphology, fruits shape, size, colours and seeds.

1.4 Genus Hippophae

The genus Hippophae Linn. consists of four species among which the most important is *Hippophae rhamnoides* Linn. The other species are *H. salicifolia* D.Don, *H. tibetana* Schlecht and *H. gyantensis*. The Indian cold desert Himalaya is represented by three

species namely *H. rhamnoides*, *H. salicifolia* and *H. tibetana* found in higher mountains of Trans-Himalaya (Chaurasia et al., 2009). Out of the three species found in India, only two species, i.e., *Hippophae salicifolia* D.Don and *Hippophae tibetana* S. are reported so far from Uttarakhand. Among them *Hippophae salicifolia* is the most common and widely distributed species. Species *Hippophae rhamnoides* L. is not found in Uttarakhand (Yadav *et al*., 2009).

1.5 The primary species that I have worked up on is *Hippophae salicifolia* D.Don.

1.5.1 Distribution of the tree

In Uttarakhand it is distributed along the river banks within an altitudinal range of 2000-3000m. In Garhwal, it occurs along Rupin-Supin, Yamuna and Bhagirathi rivers in Uttarkashi district, along Alakananda in Chamoli, Bhuyander river in valley of flower, Osla, Har ki Doon, Mallakaiphat, Garhwal (9000), Daoli, Chamoli (6500),Pandukeshwar (Badrinath), between Dyara and Dodital (Uttarkashi), Harsil and on the way to Kedarnath and Yamunotri. In Kumaon region, it occurs along Kali and Gori rivers in Pithoragarh District. In Yamunotri valley, approximate area of seabuckthorn through ocular estimation was found to be 1200 ha (Naithani and Bist, 2009).

1.5.2 Phenology

Flowering is observed from April-May. Fruiting occurs from October (Naithani and Bist, 2009).

1.5.3 Vernacular names

Known as Ames, Chuk and Chu in Uttarakhand (Naithani and Bist, 2009).

1.5.4 Description of the tree

It is a small deciduous tree up to 2.5 m girth and 10 m high with rounded spreading crown, often spinescent when young; bark reddish-brown with deep longitudinal furrows, very rough; blaze 2.5cm, fibrous, pale-yellow or yellowish-brown; twigs clotted with rusty-brown scales. With Xeromorphic structure of above ground parts of plant, it has high tolerance to atmospheric drought (Chaurasia *et al*., 2009).

Stem and Thorn

Stem is hard, woody, much branched with varied number of thorns. Branching was alternate and opposite but sometimes irregular. Each branch of stem always terminates into thorn. Thorns are appendages from stem only and are of complex type i.e. thorn were further divided into thorn (Chaurasia *et al*., 2009).

Leaves

Leaves petiole, 4-12 cm long, 0.6-1.4 cm broad, oblong-lanceolate, stellately pubescent above when young, becoming bluish green and with varying amount of pubescence when mature, canescent with dense short stellate tomentum beneath,the midrib beneath pale rusty brown with a few scales mixed wit the hairs (Chaurasia *et al*., 2009).

Fruit

Fruits are small drupe (Utricle) in all plants (Chaurasia *et al*., 2009). Raw fruits are sour to highly acidic in juice but on ripening fruits become slightly sweeter. 100 grams of fruit contains 661 fruits in average (*Chaurasia et al*., 2009). Fruits are 6.5 cm long, ovoid, yellow with scattered scales, very acid to the taste, remaining long on the trees. Fruits included within the succulent calyx-tube, drupe-like carpodermis membraneous or thin leathery, separating from combining with the seed coat, single seed with bony seed coat. The dominating fruit colour is orange and it has various shades that reach to yellow and red stages. Average weight of fruits varied from 5.6-11.0 g to 8.2-17.4 g. Fruit is a rich source of Vitamin A, B1, B2, C, E, K etc and also has tocopherols and flavanoides. Vitamin E content is higher than wheat embryo, safflower, maize and soya bean. In Vitamin C, seabuckthorn fruits are second only to Aonla (Naithani and Bist, 2009).

1.5.5 Flowering biology

Flower bud is conspicuous and stable. Flowers are unisexual and dioecious, flowering before the leaf bud burst. The male flower buds are four angled, tower-shaped, spiral tower-shaped, cruciform or bifid ovate. The female flower buds are cruciform, low spiral tower shaped, oval or bifid-ovate (Chaurasia *et al.*, 2009).

The male flowers appear from the axils of deciduous bracts, sessile, calyces almost 2-parted, 4 stamens, 2 of which alternate and the other 2 opposite with the lobes of the calyx, short filaments and anthers oblong (Chaurasia *et al*., 2009).

The female flowers are solitary, axillary or clustered into inflorescence like, short pedicles, calyces pocket like, 2 teeth lobed at apex, superior ovary with single carpel and locules and with one ovule, styles lightly exerted (Chaurasia *et al*., 2009).

Seabuckthorn is a dioecious plant where male and female plants are separate. Flowering in seabuckthorn has been generally noticed on one to two year old shoots. The flower buds are developed in the axils of leaf of each node of shoot. Plant bear local floral buds mixed with vegetative buds on shoot decreasing from base towards the tips. Under Ladakh conditions, seabuckthorn generally flowers in first fortnight of May. However, time and duration of flowering largely depends on the cultivar, prevailing weather conditions and altitude of the region. Flowering coincides with leaf expansion after breaking of the dormancy. Under Ladakh conditions, male plant flower earlier than female plants. Male plants generally flowers in end of April while female flowers appear in first fortnight of May. Male flowers produce pollen grains in abundance and they are transported in groups. It is a wind pollinated crop (Dwivedi *et al*., 2009). The germination of pollen grains occurs in 3-4 hours of pollination and fertilization take place 5-10 days after pollination (Singh *et al*., 2004, a&b)

Pollen

Pollen grains are 3 or 4 colporate, with a psilate or granulate or verrucate ornamental surface (Chaurasia *et al*., 2009).

Seed

The seeds are usually glossy, oval shaped, elliptical, rounded oval, narrow oval and egg shaped in shape and brown, light-brown to dark brown in colour. Seeds show great variations in length, thickness, seed width, seed form and mean seed weight (Chaurasia et al., 2009). All the seeds of seabuckthorn are having a sheath over them; dur to the presence of sheath, the fruits are called Utricle (Chaurasia *et al*., 2009).

1.5.6 Root

Roots are tap, much branched, having primary, secondary and tertiary root with hairs and Frankia nodules.Extensive Colonization by arbuscular mycorrhizal fungi (AMF) was studied by scientists of Institute of Himalayan Bioresources Technology (IHBT), Palampur (Gulati et al., 2001) in the young feeder roots of seabuckthorn, with typical mycorrhizal structures, such as hyphal coils, arbuscules, vescicles, extra material hyphae and chlamydospores. Noticeably formation of large number of endospores was

also observed, more frequently so in the root samples collected shortly after the melting of winter snow. Glomus spp. were found to be pre-dominant amongst the AMF associated with the plant. Ectino-mycorrhizae were not detected in the plant. The population densities of AMF spores were by far markedly higher in the rhizospheric soil as compared to the soil collected from Non- rhizospheric/ uninhabited sites. Physico-chemical analysis of soil showed that the soils were silty loam to silty clay loam in texture, alkaline in reaction, high in organic carbon and low in available N,P and K (Dwivedi *et al*., 2009).

1.5.7 Propagation through seeds

Seabuckthorn can be propagated successfully by seed which are viable for a period of two years, after which they lose viability rapidly. The seeds are small (4.5 mm in length) with brown and hard seed coat (Dwivedi *et al*., 2009). The freshly harvested seeds have a short physiological dormancy and thus cannot germinate immediately after harvesting. May is the best time for sowing of seeds in nursery beds. Before sowing, the mature seeds should be stratified for about 25 days by keeping in a box in alternate layers of moist sand. These boxes are kept under cool and shady condition for about 25 days. This method gives over 85% of germination (Dwivedi *et al*., 2001a). Seed can also be sown directly in nursery beds in the month of November where cold treatment is given before germination under natural conditions. Both male and female plants are borne from seeds. The method is useful only in raising large-scale nurseries for afforestation for conservation of mountainous wastelands. Highest germination percentage of seeds was recorded when seeds were treated with IAA @ 200 ppm followed by the treatment with GA_3 @ 400 ppm (Bisht *et al*., 2007b).

1.5.8 Propagation through hard wood cuttings

Seabuckthorn normally takes 60-75 days in rooting depending upon the soil and climatic conditions. About 85% success has been recorded in rooting of stem cuttings under Ladakh conditions These cuttings are ready for plantation in the main field in March of the following year (Dwivedi *et al*., 2006,a).

Cuttings of 1 cm diameter and 20 cm length, collected from sun facing young plants, when treated with 500 ppm of Naphthalene Acetic Acid (NAA) and planted in the nursery bed with a soil mixture of soil+sand+FYM (2:1:1), gave a high rate of plants survival (Singh, 1995). Singh et al. (1997) also found the best results of propagation in

the cuttings of 20 cm length. Aswathi *et al*. (2001) in Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, H.P., also favoured 500 ppm NAA treatment. 500 ppm NAA solution treatment to two-year-old cuttings gave the highest rooting percentages (61.10%) even after two years.

Further, propagation through Suckers, Soft wood cuttings and Micro-propagation can also be obtained, with good success (Dwivedi *et al*., 2009).

1.5.9 Germination

In some sites of Lahaul-Spiti and Kinnaur region in Himachal Pradesh, according to the studies by Singh et al. (2005), *H. tibetana* showed maximum rate of seed germination (100%) followed by *H. rhamnoides* (21-100%) and *H. salicifolia* (37%) (Dwivedi *et al*. 2009).

1.5.10 Frankia

It generally grows up to 4-5 meters below the ground bearing prominent root nodules, due to Frankia's actinorhizal symbiotic association, which is an adaptation to survive in poor soils in cold higher mountain regions (2700-3700m). The root nodules are prominent in sandy areas (Chaurasia et al., 2009). The function of Frankia is to fix atmospheric nitrogen. It is estimated that Seabuckthorn plantation fix 180kg of Nitrogen per hectare every year (Jike and Xiaoming, 1992).

Frankia is a genus of soil actinobacteria that is able to form nitrogen-fixing root nodule symbioses with actinorhizal plants (Wall, 2000). These Gram+, aerobic and heterotrophic bacteria fix nitrogen under both free living conditions and in planta inside symbiotic root nodules (Simonet *et al*., 1989; Lechevalier, 1994). The first pure culture of a Frankia strain was isolated from *Comptonia peregrina* nodules (Callahamet *et al*., 1978). Since then, several Frankia strains have been isolated from different actinorhizal species (Diem et al.1982; Diem *et al*.1983; Mansour *et al*. 1990; Gomaa *et al.,* 2008; Shash, 2009; *Singh et al.,* 2010) and are grouped into four major clusters (*Normand et al.,* 1996). Frankia strains in cluster I form nodules on members of Betulaceae, Casuarinaceae and Myricaceae. Cluster II includes Frankia strains able to infect the Coriariaceae, Datiscaceae, Rosaceae and Ceanothus of the Rhamnaceae. Frankia strains in cluster III form effective nodules with the Myricaceae, Rhamnaceae, Elaeagnaceae and Gymnostoma of the Casuarinaceae. Cluster IV includes typical frankia strains that

are non-infective either being unable to reinfect their host plant or perform non-nitrogen fixing.

1.5.11 Uses

Fruit juice is used in cardiac juice. The fruit act as an anti-aphrodisiac in female. Regular intake of fruits has a prophylactic action against cold and cough. The paste of ripe fruits along with turmeric and grain floor is externally to beautify the face of women. In Mayar and Bhanga villages of Lahaul, the traditional medicinal practitioners use fruits to treat lung disorder, blood pressure and indigestion. Preparations from the fruit berries are used to treat gum bleeding. In the dry climate of Ladakh, dried berries are used to treat cracking of lips. Fruits are also used for polishing gold and silver. The pulp remaining after the extraction of juice is used as a colouring material. Because of the high content of Vitamin C, seabuckthorn fruit is generally used to make soft drinks and other similar food products (Dwivedi *et al*., 2009). Its fruits are used for preparation of jelly that is used against pulmonary complaints. About 33.5 percent rich yellow pigment from fruits of seabuckthorn may be obtained which is widely used as a food additive (Bawa, 2004). Fruits are very perishable in nature and cannot be stored for more than 2-3 days (Dwivedi *et al*., 2009). Fruits are used by local people in the preparation of edible items like pickles, jam and as a dying ink (In Sikkim for dying blankets, bed covers and sweaters etc) (Basistha, 2009).

Its bark is an effective blood purifier and tumour inhibitor. Paste of bark is used to heal wounds and ulcer (Naithani and Bist, 2009).

Leaves are used for making green tea (Naithani and Bist, 2009). Leaves are good nutritive fodder for cattle and sheep/ goats as they are rich in protein (18-22%), fat (4-5%) and other micronutrients (Naithani and Bist, 2009). Tannin has been obtained from leaves. Leaves are rich source of polyphenols, flavanoids, anti-oxidants and dietary fibers (Dwivedi *et al*., 2009). Crude protein in *H. salicifolia* is 21.6% i.e. higher than *H. rhamnoides* (17.5-20.5%) Fat content of leaves is 4.6%. It has 5.1% ash content (Dwivedi *et al*., 2009).

Seabuckthorn oil is used to promote the healing of the skin, such as burns, sunburns, eczema and psoriasis (Naithani and Bist, 2009).

The thorny branches are used to fence the cultivated land. Its wood provides fuel and charcoal. Wood is also used for making handles for household implements

(Naithani and Bist, 2009). In Sikkim, local people use the plant as a cheap source of firewood, as per local respondents the wood of seabuckthorn burns almost with blue flame and gives a good heat with minimum smoke and ash (Basistha, 2009).

Seabuckthorn has a multipurpose uses in food and pharmaceutical industries, medicinal and aromatic factories, to high altitude farmers and researchers (Rongsen, 1992) and may be called as wonder plant of the dry temperate himalayas (Singh, 1998) due to its multilateral potentialities and utilities in ecological and economic aspects. Seabuckthorn is extensively used in 'Sowa-Rigpa', commonly known as Tibetan or Amchi medicine.

Seabuckthorn has ability to fix atmospheric nitrogen into soil (Basistha, 2001) and thus help enrich the soil.

Defence Food Research Laboratory, (DRDO), Mysore, has developed Health drink, Jam and Jelly, Yogurt, Fruit Juice powder, Herbal tea, Bakery products, Wine, Seabuckthorn oil, Food colourant and animal feed (Kumaran *et al*., 2004, 2005a)

CHAPTER-2
REVIEW OF LITERATURE

CHAPTER 2- REVIEW OF LITERATURE

2.1 Vegetative propagation

The natural habitats of seabuckthorn in Uttarakhand are generally inaccessible areas that remain devoid of human population for almost 6 months during winter, the work done on propagation and domestication of this plant in the state is negligible. It was found that when the semi-semi-hard wood cuttings which were collected in the month of November and planted elsewhere i.e. at Ranichauri (2000 amsl), the survival achieved was low at 20%. The success percentage was higher i.e., 60%, when cuttings were taken during March. On the basis of results obtained so far, it was observed that the treatment of the cuttings with IBA @ 400 ppm gives more rooting as compared to control. Best time for the collection of cuttings was found to be March (Yadav *et al*., 2006b).

Seabuckthorn can be propagated successfully by stem cuttings and this method has been recommended for commercial propagation. It helps in development of proper canopy of the plant and maintenance of male/ female ratio in the orchard. One or two year old stem cuttings having pencil thickness are ideal for its propagation. The stem cuttings are taken from identified male and female plant to get elite planting material. The best time for taking cuttings is in the month of March. The side branches and thorns are removed and 30 cm long cuttings are made (Dwivedi *et al*., 2009).

The cuttings are then placed in running water for a period of 2-3 days. Seaabuckthorn cuttings are not easy to root on their own, thus the rooting hormone Indole Butyric Acid (IBA) is applied @ 500 ppm before planting it in the nursery beds. The best time for planting of cuttings has been found to be the month of the April (Dwivedi *et al*., 2002,a).

Properly treated cuttings should be planted soon after treatment in well prepared nursery beds. Planting should be done in rows 30-45 cm apart and proper spacing between the cuttings (Dwivedi *et al*., 2004,b).

2.2 Diseases and insects/pests

During a survey of nurseries in 2005-06 in the hilly region of Uttarkhand, seabuckthorn plants with root rot caused by *Rhizotonia solani* Kuhn. were found in

Gangotri valley and in nurseries at Ranichauri. Severe mortality of seabuckthorn plants (20-25%) was observed during July - August at Forestry block of University campus. The infected plants appear sick which gradually turn yellow leading to defoliation of all leaves within 2-3 weeks. When uprooted, collar region of dead plants revealed a decay of bark and secondary roots. Roots were shredded and filled with white mycelial growth on the surface of freshly infected areas (Yadav et al., 2007).

Rhizoctonia was consistently isolated from the lesions below the bark on PDA and on water agar amended with streptomycin sulfate. Similarly aphid attack on seabuckthorn was also reported for the first time from Garhwal Himalayas during 2007 (Singh et al., 2007).

Fungal species of Fusarium, Alternaria, Pythium, Fomes and Valsa gained easy entry to even slightly damaged plants and led to various disease symptoms. Seabuckthorn is also attacked by a number of aerial or leaf soptting fungi viz. powdery mildew caused by *Phyllactinia miracula* (Paul, 1997).

Capitophorous sp. was found attacking *Hippophae salicifolia* in dry temperate Himalayas (Hameed *et al*., 1975).

Studies carried out by Bhagat et al. (2001) at Kusumeri, in Lahaul valley revealed that, the Death Hawk Moth, *Acherontia styx* (western) caused considerable damage to this plant at larval stage besides an unidentified mite species. However, some defoliating beetles belonging to sub-family Melolonthinae viz *Brahmina cariacea* (Hope), Brahmina sp. too have been found attacking Seabuckthorn. Although the attack of these beetles was sporadic, however, they reduced plant vigour and fruit bearing. *Holotrichia longipennis* Br. also caused damage to this plant at early stage of growth.

Plants of *Hippophae salicifolia* are infested by many pests like armored scale, death hawk moth, eriophyid mite, psyllid, green aphid and shoot borer. These reportedly cause medium to heavy damage to seabuckthorn plants (Sharma *et al.,* 2011; Stobdan *et al*., 2011).

Several fungal species of *Alternaria, Fusarium* and *Monilia* have also been isolated from its different parts (Singh *et al.,* 2011).

At present times, *Hippophae salicifolia* has relatively few diseases, insects and pests reported. *Verticillium* wilt in *Hippophae salicifolia* caused by *V. albb-atrum* Reinke and Berth (Laurinen, 1994). *V. dahliae* Kleb (Kennedy, 1987) attacks wide range of hosts

including herbaceous and woody species. It appears on fruiting trees aged between 5-8 years. The symptoms of yellowing and progressive wilting of the leaves, the presence of the discoloured leaves and vascular tissue are typical (Kennedy, 1987). Fusarium wilt is another important disease in *Hippophae salicifolia*. It is commonly believed that the fungus penetrates into the seedlings through wounds on the roots or stem.

Damping off of Seabuckthorn seedlings occurs frequently when cotyledons or the first true leaves open. It is believed that damping off can be caused by number of soil borne fungi, such as, Alternaria, Fusarium, Botrytis etc. Other diseases such as die back are caused by Fusarium sps (Kondrashov, 1981)

Four recently noted insects infesting the seabuckthorn are green aphid, thrips, two spotted mite and ear wigs. A few insect infestations were reported in India. Death hawk moth (*Acherontia styx* West wood) at larval stage caused considerable damage in SBT. Some defoliating beetles *Brahmina cariacea* (Hope), *Brahmina sps* have also been found attacking SBT.

CHAPTER-3
MATERIALS AND METHODS

CHAPTER 3 - MATERIALS AND METHOD

3.1 Description of source site

3.1.1 Location and Altitude

Yamunotri is situated in the western region of Garhwal Himalayas at an altitude of 3,200 m in average approximately, in Uttarakashi district, Uttarakhand state, North India.

3.1.2 Climate

It is called as Mountain climate or highland climate or alpine climate.

According to Koppen, climate of Yamunotri is classified as '*Cfb'*. *Cfb* **(**Mountain climate**)** in India is closely explained as follows: In the Himalayan mountain, the temperature falls by 0.6^0 C for every 100 m rise in altitude and this gives rise to a variety of climates from nearly tropical in the foothills to tundra type above the snowline. One can also observe sharp contrast between temperatures of the sunny and shady slopes, high diurnal range of temperature, inversion of temperature and variability of rainfall based on altitude.

The northern side of the western Himalayas also known as the trans-Himalayan belt is arid, cold and generally wind swept. The vegetation is sparse and stunted, as the rainfall is scanty and the winters are severely cold. Most of the snowfall is in the form of snow during the late winter and spring months. The area to the south of the Himalayan range is protected from cold winds coming from interior of Asia during winter. The leeward side of the mountains receive less rain while the well exposed slopes get heavy rainfall. The places situated between 1070 and 2290 m altitudes receive the heaviest rainfall and the rainfall decrease rapidly above 2290.

The great Himalayan range witness heavy snowfall during the winter months of December to February at the altitudes above1500 m. The diurnal range of temperature is also high. The states of Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Arunachal Pradesh and Sikkim experience this kind of weather.

3.1.3 Topography

The Yamunotri region is characterized by a hilly terrain and consists of many glacial lakes, snow-fed streams, glaciers, high snow clad peaks of the Himalayas, densely forested low lying hillocks covered mainly with Tropical & Alpine type forest covers.The average height of Yamunotri from the sea level is 3955 meters and its latitudinal and longitudinal extents are **31°01′N 78°27′E** & **31.01°N 78.45°E.**

3.1.4 Soil type of Yamunotri

It is called Mountain and hill soils. It is a collective name given to a number of soil types occurring at very high altitude areas under sub-tropical and temperate conditions under a wide variety of forests. These soils have been termed ass ***Dabar soils*** in some parts of Gaarhwal-Kumaon .Mountain and hill soils are very thin, fertile and may be less than a centimeter deep on slopes. These may occur at elevations above 2750 m in the western Himalayas, over 2500 m in the Nepal and 2400 m in the Eastern Himalayas. These soils are mixed with pebbles and gravel in many regions. The texture varies from sandy to sandy loam. The soil reaction varies from acidic to neutral and the organic matter content of these soils ranges from 1 to 5 %.

3.1.5 Description of Ranichauri work site i.e. Forest Nursery

The experiment site College of Forestry,V.C.S.G. Uttrakhand University of Horticulture and Forestry, Ranichauri, Tehri Garhwal, is located 10 km away from Chamba (Rishikesh-Gangotri Road) at an altitude of about 2100 m mean above sea level, lying between 30^0 15’ N latitude and 78^0 30’ E longitudes under mid hills of Uttrakhand, India.

Major objectives of the study will be as follows:

1. Identification of pathogens affecting the vegetative propagation of *Hippophae salicifolia* D.Don semi-hard wood cuttings in Ranichauri Forest nursery.
2. Identification of insect pests/mites/natural enemies of pests on *H. salicifolia* in Yamunotri provenance of Uttarakhand.

3.2 IDENTIFICATION OF PATHOGENS AFFECTING THE VEGETATIVE PROPAGATION OF *Hippophae salicifolia* D.Don SEMI-HARD WOOD CUTTINGS IN RANICHAURI FOREST NURSERY.

Methods in forest diagnosis

Knowledge of general laboratory techniques consisting of preparation of slides, section cutting preparation of media, methods of sterilisation, isolation, pure culture and inoculation are pre-requisites. Diagnosis is generally done in four ways.

3.2.1 Direct observations

Careful examination of diseased specimens in the forest often allows preliminary diagnosis and aids selection of material for subsequent laboratory investigation. The following items are required for investigation;

Hand Lens

X 15 is the best all round choice- sufficient to see many sporolating structures of fungal parasites on tree.

Knife

The most useful is one with sharp finely serrated blade allows fairly tough stem and root tissues to be open to check for internal symptoms.

Trowel: A trowel with 15 cm blade is essential for sampling root systems, tubers, soil etc

Sample containers

Brown paper bags or envelopes are best for most purposes and can be easily written on using a pencil or water proof pen; polythene bags are suitable only for short term storage of diseased specimens as they encourage condensation which promotes growth of surface saprophytes, especially in tropics. Specimen tubes like plastic universal bottles are ideal for smaller specimens or those which are very delicate.

Camera:

A macro-lens or other device for taking close up photographs is usually needed for detail of localized symptoms, on-site records of gross symptoms and their distribution.

Recording materials

Notebook, labels, indelible markers etc for field records of observations and samples.

3.2.2 Moist chamber or BOD Incubator:

Moist chamber or BOD Incubator is used to incubate infected tissue to stimulate the growth of associated pathogen. For the preparation of moist chamber, take a clean glass Petri plate and round blotting paper. Place one in lower lid and another in upper lid of the Petri plate and moisten them. Take a piece of infected part and keep it on the slide and close the upper lid. Then incubate the plate at room temperature (usually 26-28 degree celsius) for one or two days. During the period the fungus will grow on the infected parts which can be observed under microscope.

3.2.3 Microscopy

Microscope is the scientific equipment that is frequently used for studying microorganisms and structures too small to be examined properly with naked eye.In the pathology lab, Binocular compound microscope is used with maximum magnification of 40X.

Procedure for using microscope

- For maximum effectiveness, the mechanical and optical components of the microscope must be clean and aligned and following procedures should be practiced each time the microscope is used.
- Wipe the ocular, objective, surface of condenser and mirror with a lens tissue. If they are heavily marked, moisten the lens with a little alcohol and wipe the surface.
- Check that the diaphragm is fully open and condenser is completely racked up.
- Check that the slide preparation is dust and finger print free. Wipe, if necessary.
- Place the specimen slide on the microscope stage and focus on the specimen.
- Always use the fine adjustment when using the high power. Try not to let the objective come into contact with the slide. This may damage the slide or the objective.
- Ensure that the low power lens is in place while placing or removing a slide on stage.

Scrape

The scrape taken from the infected area is observed under microscope for the presence of the fungal structures.

Thin sections

Thin sections of the infected area, transverse or longitudinal, are made and observed under microscope for the presence of the fungal structures. Small pieces of plant tissue, surface scraping or section can be placed directly onto a glass microscope slide in a drop of water and covered with a cover slip. This simple technique allows a preliminary observation for the presence of some pathogens and is useful for detecting bacterial streaming from lesions caused by bacteria. Lactophenol is a good mounting and clearing medium for diseased plant tissues.

3.2.3 Lacto-Phenol Blue Staining:

Lacto-Phenol Blue Stain stains the fungal cytoplasm as light blue region thus it provides a light blue background against which unstained hyphal walls can easily be seen under microscope. Fungal cell being large in size can easily be observed even without staining. However, staining help in observation of other structures of the fungal cell.

Objectives

To observe various components of fungal cell like hyphae, sporangia etc, under the Microscope.

Materials required

Young cultures of fungal species that are to be identified.

Procedure:

- Place a drop of lactophenol blue in the centre of a clean side.
- Sterilise the inoculation needle on Bunsen flame and allow it to cool. Transfer a little of fungal mycelium mycelium into the lactophenol blue on the slide.
- Using two mounted needles, tease out the hyphae so that the hyphae are separated, which will help in the observation of individual hyphae.
- Cover the preparation with a thin cover slip carefully so that air bubbles are not trapped inside. This can be achieved by placing one edge of cover slip on the slide and lowering the cover slip over the preparation slowly with the help of a

mounted needle. In this fashion when needle is removed the cover slip will fall on the preparation with exclusion of air.

- Examine the slide using low-power and high-dry objectives under binocular microscope.

Results

The fungal cytoplasm is seen as lightly stained blue region inside the unstained hyphal cell wall.

Observations

Examine and look for the type of hyphae (septate or aseptate) and sporangia. Also note the type of spore and draw diagrams of hyphae, sporangiophores and spores in your note book.

3.2.4 ISOLATION OF THE PATHOGEN:

The separation of a pathogen from its host and its culture on a nutrient medium is called Isolation. Diagnosis of a particular disease and isolation of the pathogen is required for identification of the pathogenic agent.

3.2.4.1 Isolation of Fungal species that effect the growth of *Hippophae salicifolia* D.Don.

Objectives

To isolate Pathogenic agents from the infected parts of *Hippophae salicifolia*

Materials required

200 g Potato, 20 g Agar, 20 g Dextrose, Distilled water, Antibiotic (Streptomycin)

Procedure

Collect the infected portion of plant like Root, Leaves, Stem, etc and cut 5-10 mm^2 sections from the margin of the lesions, in the transition tissues between healthy and dead tissues.

- Arrange four sterile Petri dishes in a row near the flame.
- Fill Mercuric Chloride solution in the first plate.
- Pour aseptically sterile water in the rest of the dishes.

- Small sections of tissues are placed in mercuric chloride solution for 1-2 minutes.
- Using a sterile forceps, wash the sections in two changes of sterile water.
- Thereafter, the sections should be blotted dry on sterile paper.
- After blotting dry, place 2-4 surface sterilized bits on PDA plate.
- Incubate at 25° C for growth.
- After 72 hours, examine the fungal growth association with the bits and aseptically transfer to PDA slants to get a pure culture.

Results

Well-grown mycelial mass will appear in 2-4 days.

Observations:

Look for the fungal sporangia and hyphae (septate or aseptate) under microscope after Lacto-Phenol staining the fungi from the pure culture on successive days.

Compare the hyphal and sporangia characters and microscopic details of the fungi with the authentic description for identifying the fungi.

3.2.4.2 CULTURE MEDIA

To cultivate microorganisms in the laboratory, we must fulfil their nutritional requirements. Like all other living beings, microorganisms need (i) water, (ii) a source of carbon, which also acts energy source, (iii) some inorganic compounds. These substances which are required for the growth of microorganisms are termed Nutrients. When micro-organisms are grown in the laboratory, it is a normal practice to obtain them in pure culture. Micro-organisms require nutrients for their growth, development and biological activities. A balanced chemical composition substance or solution used for the culture of micro-organism is called Culture Medium (Plural- Media).

A cultural medium of solid media type consistency was prepared for the isolation of fungal pathogens:

- When preparing a media, it is helpful to remember the following points:
- Fungi usually grow best in a rich carbohydrate medium.
- Fungi usually prefer a slightly acid reaction, pH 6-6.5, while bacteria prefer pH 7.
- Media should not be over sterilized.

- Agar is slow to dissolve thoroughly (1-2 hr boiling).
- Agar does not solidify satisfactorily in very acid or alkaline solutions.
- For fungi isolation, at the time of pouring, add antibiotic to prevent bacterial contamination.
- Peptone may generally be omitted from fungus media.
- Tap water is often preferred to distilled water as it contains useful trace elements.

Media used for fungal isolation:

Potato Dextrose Agar Medium (PDA)

Scrub the potato clean, do not peal and cut into 12mm cubes. Weigh out 200g, rinse rapidly in running water, place in 1 litre water and boil until soft. Strain mesh and squeeze as much as pulp as possible through cheesecloth. Add 20 g of Dextrose and 20 g Agar and stir till dissolved. Make up to one litre and sterilize at15 p.s.i for 20 minutes.

3.2.4.3 Solid Nutrient media in Petri dishes

Once the medium has been selected, the ingredients are measured in to 1 litre flasks in an amount to yield 500 ml with distilled water. The flasks should be no more than half full to prevent boiling over in the autoclave. Autoclaving will dissolve and disperse the ingredients.

After complete sterilization of media in an autoclave and Petri dishes in an oven, the medium is poured into glass/disposable plastic Petri dishes to a depth of about 5mm. All the following activities are followed under laminar air-flow.

- Wipe the table top with rectified spirit.
- Put on U.V lamp on for 5 minutes.
- Wash hands with rectified spirit and air dry.
- Light the burners.
- Arrange sterile Petri dishes near the burner.
- Hold the flask with warm PDA and remove the cotton plug near the flame.
- With left hand, raise one side of the Petri plate cover just enough to insert the mouth of the flask and pour 20 ml of medium.

- Replace the cover immediately and gently rotate the dish so as to spread the medium evenly.
- Hold open mouth of the flask near the flame and retain the plug in the left hand. Similarly pour some Petri dishes with medium.
- Allow the plated to cool sufficiently for the medium to harden, before using.

3.2.4.4 Solid Nutrient media in test tube slants

An agar slant is similar to that for Petri plates. The major difference is that the medium is heated to dissolve the ingredients and then poured about one third fill into test tubes. The test tubes are then plugged with non-absorbent sterilised cotton and autoclaved. The culture tubes with medium are placed in a slanting position on wooden blocks to set the agar in a slanting position. The slanting medium allows more surface area on which inoculated microorganisms can grow.

3.2.4.5 CONTROL OF MICROBIAL GROWTH BY STERILISATION

Sterilisation is the process that makes the materials/objects free from all living organisms. Microbial study with pure cultures is impossible if the glass ware and culture media were contaminated with any other kind of organisms at the time of use. Thus the test tube flasks, pipettes, Petri plates, culture media etc must be sterilised before use. Once sterilised, media or glassware must be maintained in sterile condition indefinitely by protecting them from atmospheric contamination. This is ensured by plugging the test tubes, flasks, bottles etc with cotton and placing the pipettes, Petri plates etc in aluminium/stainless steel/copper container before sterilisation. This will protect these materials from dust and outside contamination after sterilisation.

Sterilisation is achieved by exposing materials to lethal agents which may be chemical, physical or ionic in nature or in the case of liquid, physical elimination of cells from the medium. The methods commonly used for sterilisation are heat, gas and in the case of liquid, ultra filtration. Inoculating needles are sterilised by heating on flame by redness.

In the pathology lab, moist heat is commonly used. Moist heat is usually provided by saturated steam under pressure in an autoclave or pressure cooker. Under pressure, moist media can be sterilised by heating at 10-15 p.s.i or under metrication one par gauge pressure for 15 min in an autoclave or domestic pressure cooker. Moist heat has

advantage over dry heat as conduction of heat is rapid and has greater penetrating power. The temperature required for sterilisation is lower and the duration of exposure is shorter. For most purposes 15 min. at 121°C or 30 min. at 115 °C are recommended.

To use the autoclave

- Check that sufficient water is present and load the material to be sterilised.
- Screw down the lid; tighten diametrically opposite wing nuts in pair so that the lid is clamped evenly on the gasket.
- Open steam cock and light gas or switch on electricity.
- Let steam issue for at least 5 min. to remove all air before closing cock. This is very important to obtain correct temperature.
- Adjust pressure and turn down gas.
- Allow operating for the required time and turn out gas or electricity.
- Let pressure drop to zero by cooling, then open steam cock.
- Wait for 5 min and then open lid.
- Ultra violet radiation is used to kill the microorganisms in the air. UV sterilisation is a favourite method for sterilisation of large surface area or air space. UV radiation has poor penetration because it is strongly absorbed by media components. UV radiation is used in Laminar flow, inside which most of the laboratory works are carried out.

Surface sterilisation

Surface sterilisation of plant material is effected by immersion in 1:1000 solution of mercuric chloride for 5 min., and then washing in several changes of sterile water. Chromium plated or stainless steel instruments such as scapels, forceps, scissors etc. should be dipped in alcohol and flamed. Sodium Hypochlorite (concentration: 0.525%, exposure time: 2-5 minutes) is widely used as surface sterilisation.

MATERIALS USED

Instruments

Binocular Compound microscope, Laminar airflow, Freezer, Incubator, Autoclave, Pressure cooker, Micro wave Oven.

Tools

Spirit Lamp, Forceps, Inoculation needle, Sterilised non-absorbent cotton, Cover slides, Slides, Cotton plugs, Blade, Knife, Container bags, Zip-up bags, Note pad, Note book, Markers, Pen, Pencil, Scale, Petri plates, Petri dishes, Conical flasks, Test tubes, Beaker, Paraffin film, Match box.

Chemicals

Sodium Hypochlorite, Ethyl alcohol, Rectified spirit, Streptomycin, Potato Dextrose Agar, Nutrient Agar, Distilled water.

3.2.4.6 To isolate and identify the pathogen associated with the mortality of the cuttings

Instruments

Binocular Compound microscope, Laminar airflow, Freezer, Incubator, Autoclave, Pressure cooker, Micro wave Oven

Chemicals used

Indole Butryic Acid (IBA)

Materials required

Secateur, Poly bag, Gunny bag, Knife, Sickle, Thread, GPS, Rust-Proof metal Tags, Indelible Markers, Red paint, Paint brush, Notebook, Pen, Pencil

Tools

Spirit Lamp, Forceps, Inoculation needle, Sterilised non-absorbent cotton, Cover slides, Slides, Cotton plugs, Blade, Knife, Container bags, Zip-up bags, Note pad, Note book, Markers, Pen, Pencil, Scale, Petri plates, Petri dishes, Conical flasks, Test tubes, Beaker, Paraffin film, Match box.

Chemicals

Sodium Hypochlorite, Ethyl alcohol, Rectified spirit, Streptomycin, Potato Dextrose Agar, Nutrient Agar, Distilled water.

3.2.4.7 Pre-requisite criteria for selection of trees to be considered for collection of semi-hard wood cuttings for propagation

- Trees should have acceptable morphological characters like distinct stem, well developed crown, good vigour, usually at least similar or better than the representative of the mature, middle-aged trees in the given provenance.
- Trees should be possibly not or least infected/infested by diseases/pests.
- Trees should not be too young or over mature.
- Trees should have satisfactory flowering and fruiting.
- Trees should not be stunted, malformed, crooked, a whip tree or a wolf tree.

3.2.4.8 Pre-requisite criteria to be considered for the selection of semi-hardwood cuttings for vegetative propagation

- The cuttings should be collected from healthy, disease and pest free trees.
- The cuttings should have pencil size thickness (5-10 mm). The cuttings with larger thickness have shown poor success.
- The cuttings should be straight and free of damages like cuts, bruises, wounds etc
- In case of *Hippophae salicifolia*, the cuttings should be collected in the months of March or April, after which there is bud initiation resulting in failure of the cuttings.
- Semi-hard wood cuttings should be used and can be checked by a small test. The cutting is bent into semi-circle, gripping between the thumb and index finger. If the cutting is too immature, it folds in the middle with out attaining the normal straightness. If the cutting is too woody, it breaks in the middle. The right cutting comes back to straightness after released from the bending force of hands. In case of *Hippophae salicifolia*, the cuttings are quite woody in nature.
- The cuttings should have at least 2-5 live bud nodes above the 1/3rd basal portion of the cuttings (because it is covered by rooting media when planted).
- The cuttings with excessive bud initiation prior to collection should not be considered for propagation.

3.2.4.9 Procedure for collecting and handling semi-hard wood cuttings prior to planting

- Firstly, the mother trees in their natural habitats are selected based on certain criteria.
- Once the mother trees are selected, they are marked with a '+' (cross-like) mark on their stem (above 0.75-1 m from the ground level) using a red water proof paint.
- The marked tree is tagged with a rust proof metal tag having descriptions like date of collection, collector, provenance, etc. Indelible markers are used to write on the metal tags.
- After marking and tagging is done, cuttings relatively of pencil size are collected by giving a clean cut across the plant twigs, using a clean, well maintained, sharp secateur.
- As soon as the cuttings are collected from individual tree, they are tied into the bundles using a thread and are tagged by metal tag with the descriptions.
- Then, the bundles are immersed in running water if there is available water source near by or simply placed inside a wet gunny bag or a poly bag after moistening them with water, for transportation. This is done to prevent loss of moisture from the cuttings (leads to drying up and mortality).
- Care should be taken to not keep the cuttings under sun or in warm places during collection or transportation. The bundles should be kept in shade or at normal room temperature as much as possible.
- Once the cuttings are bought to location of planting field from their natural habitat, they are removed from gunny bags and are partially immersed in the containers having clean tap water. The water level in container should be such that only 1/3rd of the basal portion of the cutting is immersed in water.
- Partial immersion should not be carried out longer periods (not more than 12-24 hrs). It may result in rotting of the propagation material or cut ends of cuttings. Partial immersion is necessary to bring the water potential through out the cuttings to equilibrium.
- The cuttings after immersion-treatment should be immediately treated with rooting hormones prior to planting.

3.2.4.10 IBA (Indole Butyric Acid) Treatment to semi-hard wood cuttings prior to planting

IBA (Indole Butyric Acid)

Also known as Indole-3-butryic acid. Molecular formula is $C_{12}H_{13}NO_2$. IBA is a plant hormone in the auxin family. IBA is used to initiate root formation. The peroxisomal IBA to IAA conversion is followed by peroxisomal Nitric oxide, which is important for IBA induced lateral root formation (Schlicht *et al*., 2013).

ppm (parts per million)

ppm is used to measure concentration, usually in a solution of water**.** ppm is a value that represents the part of a whole number in units of 1/1000000. 1 ppm is equal to 1/1000000 of the whole. (rapidtables.com)

1ppm = 1/1000000 = 0.000001 = 1 $x10^{-6}$ = 0.0001%.

Experimental Treatments

In an experiment, the factor (also called as independent variable) is an explanatory variable manipulated by the experimenter. Each factor has two or more levels i.e. different values of the factor. Combinations of factor levels are called Treatments.

In the experiment that is to be carried out, there are 3 treatments stated below:

T_1 = Treatment 1 - 100 ppm of IBA

T_2 = Treatment 2 - 250 ppm of IBA

T_3 = Treatment 3 - 400 ppm of IBA

3.2.4.11 Preparation of rooting media for semi-hard wood cuttings

Rooting media/ substrates

In botany, a rooting medium is any type of substrate that encourages root growth**.** This substrate normally comprises of different organic components and minerals. It usually acts as a store house of water, air and mineral supply ensuring easy rooting of cuttings and their further growth. Rooting mainly depends upon the species usually.

Rooting trays

It is a container with a drainage hole at the bottom. It aids rooting of the cuttings, achieving the development of dense root system. It prevents the coiling of roots.

Following rooting substrates were used:

S_1 = Sand

S_2 = Soil

S_3 = FYM

S_4 = Soil + Sand + FYM (2:2:1)

S_5 = Mother soil

S_6 = Mother soil + + Soil + FYM (1:1:1:1)

S_7 = Mother soil + Soil + Sand + FYM (1:1:1:1)

S_8 = Mother soil + Soil + River Bed Sand Gravel + Horse manure +Litter manure (1:1:1:1:1)

Guidelines to plant the cuttings in the rooting media:

Spacing

5cm x 5cm is required.

Depth

The cuttings should be planted such that not more than 1/3rd of the length of the cutting should be covered by the substrate. Hole is made using a pencil like stick with a sharp end.

Irrigation

Water has to be given regularly on daily basis, avoiding the peak sunshine hours. Watering should be done carefully not exceeding the carrying capacity of the soil, as it may result in rotting of the exposed cross-sectional basal cambium ring from which the lateral roots arise.

Observations

Reading interval = 1 month

Bud initiation and Rooting (3months)

The number of cuttings that showed marked bud initiation (after planting in rooting trays)

3.3 IDENTIFICATION OF INSECT PESTS/MITES/NATURAL ENEMIES OF PESTS ON *Hippophae salicifolia* IN YAMUNOTRI PROVENANCE OF UTTARAKHAND

(According to the guidelines of Zoological Survey of India)

The World of Insects presents a fascinating panorama of living natural resources. Insects outnumber, in today's world, all other living forms and can be said to represent the perfect adaptation in all possible ecological niches. The geographical location of India between 8°4'N and 36°6'N, provides a wide latitudinal extent, offering a range of climatic conditions. The altitudinal variations along with other biotic and abiotic factors lead to an array of insect-faunal elements.

India can be divided into 9 major floristic regions (Yamunotri comes under Western Himalaya region including Kashmir valley, Simla hills, Kumaon hills, Garhwal hills and Doon valley) and till now some areas under each region remain moderately explored or unexplored.

3.3.1 COLLECTION OF INSECTS

Collections, once made, are to be preserved in a manner which provides scope to examine the specimens for identification and study and also guarantees long period of storage, with proper care. Scientists, going to the field for faunistic survey, may conveniently collect samples in vials and bring them to Head Quarters for extraction. Attention should be paid not to stuff too much of samples in one vial, so that the sample does not get pressed or crumbled.

Samples supersaturated with moisture should be aerated intermittently by opening the mouth of each vial. Otherwise, the evaporation of moisture and subsequent condensation may result in the formation of water droplets on the wall of the vial in which small organisms like Corrembola, mites, etc. may get trapped and die. Locality and other ecological details (for example, nature of terrain etc.) should be written in pencil on a piece of paper and dropped inside each vial containing sample.Insects live in diverse habitats and to collect insects during field survey, one has to use a variety of methods. Hand picking is found to be the best method for a geographical terrain like Yamunotri. Shaking, with a cloth under the plant part is also useful.

Hand picking

Small insects, specially the soft bodied ones are best collected by hand either with the help of a fine camel hair brush or by a forcep. The soft brush is normally dipped in the medium in which the insects are going to be preserved, so as to minimise the damage to soft skin. Forcep can be used carefully to avoid damage to the insect as in the cases of ants and many insect larvae. Hand picking needs searching in particular habitat and as such offer excellent data for biology. Insects like leaf-miners (Diptera), aphids (Hemiptera), bark inhabiting beetles, insects living under stone and vegetable (Dermaptera and Coleoptera), termites and ants etc. are only collected by hand picking.

3.3.2 EQUIPMENTS

Killing Bottles

Killing bottles are used to kill and preserve insect without affecting its colour. Usually glass jar with a layer of cyanide covered with plaster of paris, is used as a killing bottle. Cyanide vapour however may make small specimens brittle andeven Change its colour. Very often some other liquid chemicals like Chloroform, Benzene, Ether, Carbon Tetrachloride are used. Each one of these has some disadvantage but in general their vapours serve the purpose of killing the insects. The liquid may be poured over a layer of cotton and one or two filter paper or blotting paper could cover the soaked cot ton and also prevent the specimen from coming in direct contact with cotton. Insects must be handled carefully while they are put inside the bottle or taken out to prevent damage. A killing bottle with a layer of small chips and saw dust which is soaked with a few drops of ethyl acetate serves satisfactorily for a number of insects in killing and preserving the specimen which remain flexible.

Collection vials: Small specimens, which are killed and preserved in liquid, are to be kept in Homeopathic vial or similar other vials. Vials with screw plastic caps are preferable. These maybe numbered beforehand and the corresponding number in the field note book may contain details about particular collection.

Hand Lens

A lOX hand lens (folding type) is useful to examine material in the field and should be kept handy.

Paper packets

Paper packets are used to keep Lepidopteran, Odonates and many other insects. As soon as they are killed specimens are transferred to these packets, made up of oil-paper, for temporary storing and transportation. These may be prepared in desirable sizes before one proceeds for field collection.

Chemicals and cotton

During field collection, preservative like90% alcohol, killing agents like benzene or chloroform, or ethyl acetate should be carried extra, to meet any emergency; cotton may be required for packing after collection or to change the killing agent in the killing jar and should be kept handy.

Camera

A field camera with f. 1.4 or f. 1.8 lens and a set of close-up accessories would be very useful for photographing ecological conditions, insect community, feeding site, habitat and other observation.

Field Note Book

A field note book is most essential for keeping all the data. Generally a numbered tag may be attached with the collection and the same number in the field note book may be used to keep the following data: i) Date of collection (ii) Place of collection - indicate direction, approximate distance in km. from nearest road head and altitude (iii) Habitat (iv) Live colour (v) Name of host plant or animal (vi) Associated insects or animals (vii) Name of collector etc. A general note on the collection locality would provide further information, say about a Reserve Forest area, its vegetation type etc.

3.3.3 PRESERVATION OF INSECTS

After the collection is brought to the laboratory, it has to be made ready for study and permanent storage. First step in this direction is to relax them in a proper relaxing box. A cotton bath soaked in mixture of camphor and carbolic acid with a ratio of I : 3,

should be put in relaxing box to prevent moulds and fungi. Then sufficiently relaxed, the insects are pinned and set.

Proper labelling of the specimens is of extreme importance. For dry as well as wet specimensch data labels are to be written on good quality bond paper with Indian ink. Data label should contain the name of locality, altitude, name of host plant (if known), date of collection, name of collector, etc. Label should be small (lO x 20 mm.), clean and uniform.

Soft bodied insects are preserved in air tight containers having 80% alcohol in it. Label has to be stuck on the container.

3.3.4 IDENTIFICATION OF INSECTS

Identification of insects always demand well preserved specimens and reference collection to compare with. Useful taxonomic literature along with reference collection on the group can be said to be the keystone for any short-term or long term entomological work. and Packing and transporting or mailing insects need special care.

The collected, insect specimens would be sent to Entomology Division, GKVK, Bengaluru, Karnataka, for identification. Care is taken to pack the specimens with utmost precaution, so that samples reach the experts without any damage.

3.3.5 Field methodology

The terrain of Yamunotri, especially around Janakichatti is having steep slope, loose-weathered soil and sparse distribution of *Hippophae salicifolia*, generally. Hence it is very difficult to apply any scientific model or methodology, especially when the research group has a single person. So, the observer i.e me itself, chose to do Perambulation Survey in the area having population of *Hippophae salicifolia*, which involves moving along a line from a starting point, beginning through the road edges, then slopes and finally along the river bed and arriving at a different end point till the terrain is accessible. The length of the survey line depends upon the accessibility. Young seedlings were to be neglected for taking readings and about 100 middle aged to old aged trees were considered for observation and collection of insects or other diseases.

CHAPTER- 4

RESULT AND DISCUSSION

CHAPTER 4- RESULTS AND DISCUSSION

4.1 Identification of pathogens affecting the vegetative propagation of *Hippophae salicifolia* semi-hard wood cuttings in Ranichauri forest nursery

Fungi vary widely in size and shape, from unicellular, microscopic organisms to multicellular forms easily seen with the naked eye. Individual cells range from 1 μ to 30 μ. Microscopic fungi exist as either molds or yeasts or both. Internally, fungal cells are fairly typical eucaryotic cells. Fungus is a member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, Fungi, which is separate from plants, animals, protists and bacteria. One major difference is that fungal cells have cell walls that contain chitin, unlike the cell walls of plants and some protists, which contain cellulose, and unlike the cell walls of bacteria. They accomplish this by growing through and within the substrate on which they are feeding (Gilman, 1957)

4.1.1 FUNGUS ISOLATED FROM CAMBIUM: 'C1'

The fungus was isolated from the cambium portion of root of the infected seedlings. The cambium was observed to be infected at the collar region of the four month seedlings. Three infected seedlings, almost dead by yellowing of leaves and marked discolouration of collar region were bought from the field nursery to the lab. The upper branchy parts of seedlings were cut at about 15 cm from the base of the collar region. The stem along with the root system was washed with tap water to remove the soil and dirt from the root surface. Then treated with 5% of Sodium Hypochlorite solution for a minute in a bowl and then washed with sterlised water in sets of three petri dishes, successively from one after the other. Then the discoloured, rotting, infected small tissue from the cambium of the collar region and was sub cultured in PDA under controlled condition at 24-27^{0}C in BOD.

The fungus started growing with the production of white cottony mycelium after 4 days from the date of inoculation of fungus onto the PDA. The fungus grew radiating outside in a circular manner. After 5 days, the colony was clearly observable as small irrecular circle of white hyphal mass. Contamination was observed via another dark

pigmented fungus, growing in the shape of ovular rings at the periphery of the petri dish. Further the culture was purified to get pure culture. The pure culture developed fully covering the petri dish by 24 days from the day of inoculation for pure culture. The rate of growth was observed to be slower than other isolated lab grown fungal species of agricultural importance i.e 14-18 days. On the 27th day onwards, the fungal surface at the central portion of the colony started producing lightly yellow colored honey dew like droplets, showing the signs of of initiation of sporolation as observed in many dew secreting fungal species. The specimen was inoculated to the test tube slant of PDA on 29th day of pure culture isolation and was kept in BOD for a week. After that the slant was safely packed and sent to Indian Type Culture Collection (ITCC), New Delhi, for identification.

4.1.2 FUNGUS ISOLATED FROM ROOT- 'R1'

The fungus was isolated from the tap root portion of the infected seedlings. The root was observed to be infected at the collar region of the four month seedlings. Three infected seedlings, almost dead by yellowing of leaves and marked discolouration of collar region were bought from the field nursery to the lab. The upper branchy parts of seedlings were cut at about 15 cm from the base of the collar region. The stem along with the root system was washed with tap water to remove the soil and dirt from the root surface. Then treated with 5% of Sodium Hypochlorite solution for a minute in a bowl and then washed with sterlised water in sets of three petri dishes, successively from one after the other. Then the discoloured, rotting, infected small tissue from the root was sub cultured in PDA under controlled condition at 24-27^{0}C in BOD.

The fungus started growing with the production of white cottony mycelium after 3 days from the date of inoculation of fungus onto the PDA. The fungus grew radiating outside in a circular manner. After 2 days, the colony was clearly observable as small irregular circle of white hyphal mass. Contamination was observed via another dark pigmented fungus, growing in the shape of ovular rings at the periphery of the petri dish. Further the culture was purified to get pure culture. The pure culture developed fully covering the petri dish by 16 days from the day of inoculation for pure culture. The rate of growth was observed to be faster similar to other lab grown fungal species of agricultural importance i.e 14-18 days. There was absence of dew like secretions on the hyphal mass even after 20 days. The specimen was inoculated to the test tube slant of PDA on 21st day of pure culture isolation and was kept in BOD for a week. After that

the slant was safely packed and sent to Indian Type Culture Collection (ITCC), New Delhi, for identification.

4.1.3 FUNGUS ISOLATED FROM ROOT-‘ R2’

The fungus was isolated from the secondary root of the infected seedlings. The root was observed to be infected at the root region of the four month seedlings. Three infected seedlings, almost dead by yellowing of leaves and marked discolouration of root region were bought from the field nursery to the lab. The upper branchy parts of seedlings were cut at about 15 cm from the base of the collar region. The stem along with the root system was washed with tap water to remove the soil and dirt from the root surface. Then treated with 5% of Sodium Hypochlorite solution for a minute in a bowl and then washed with sterlised water in sets of three petri dishes, successively from one after the other. Then the discoloured, rotting, infected small tissue from the secondary root region was sub cultured in PDA under controlled condition at 24-27^{0}C in BOD.

The fungus started growing with the production of white cottony mycelium after 1 day from the date of inoculation of fungus onto the PDA. The fungus grew radiating outside in a circular manner. After 2 days, the colony was clearly observable as small irregular circle of white hyphal mass. The pure culture developed fully covering the petri dish by 12 days from the day of inoculation for pure culture. The rate of growth was observed to be fastest among the three species isolated. There was absence of dew like secretions on the hyphal mass even after 20 days. The specimen was inoculated to the test tube slant of PDA on 21st day of pure culture isolation and was kept in BOD for a week. After that the slant was safely packed and sent to Indian Type Culture Collection (ITCC), New Delhi, for identification.

All together out of three fungal pathogens isolated i.e. C1 (isolated from cambium), R1 (Isolated from primary root) and R2 (isolated from secondary root), R2 seems to the fast growing species followed by R1 and C1. Only C1 produced honey dew like droplets on the surface of hyphal mass and showed slight alteration of color from brilliant white to slightly yellowish white. Though the damage and effect of these fungal species on the vegetative propagation of Hippophae cuttings of four month experiment period could not be quantified because of the complexity of the diseases like the symptoms where rotting of the cut end cambium of the cuttings and swelling up of the base due to the food photosynthesized by the bud-leaves in the initial two months, yellowing of leaves

followed by fall of leaves in two-three month old cuttings and ultimately death of four month rooted cuttings was observed due to rotting of the roots. Only about 4.51% of the 288 cuttings survived as a healthy five month old seedlings. Even though all the failed rootings are not because of the soil borne fungal pathogens, it is sure that these cause mortality of cuttings in combination with other physiological and genetical aspects of the Hippophae cuttings. The variation of rooting media (8 substrates) or the rooting hormone concentrations (3 ppm concentrations) did not influence any advantage for the Hippophae cuttings for healthy and infection free growth.

4.2 IDENTIFICATION OF INSECT PESTS/MITES/NATURAL ENEMIES OF PESTS ON *Hippophae salicifolia* IN YAMUNOTRI PROVENANCE OF UTTARAKHAND

Based on the field survey conducted in the *Hippophae salicifolia* jungles of Yamunotri region during the time of late March, following insects and mites were collected, recorded and identified.

4.2.1 PREDATORY MITES

4.2.1.1 Introduction

Mites comprise the Acari, which are the largest group within the arthropod class Arachnida, with over 48,000 described species. In contrast with other arachnid groups such as spiders and scorpions, mites are distinctive in both their small size (adult body length ranging from 0.1 to 30 mm) and their ecological diversity. Some mites are predators, like almost all other arachnids, but mites may also feed on plants, fungi, or microorganisms or as parasites on or in the bodies of other animals. Mites are among the oldest known groups of arthropods, with a fossil record beginning in the Devonian period (Connor, 2009).

Contrary to the injurious mites, there are some beneficial mites too which act as our friends by predating upon phytophagous mites and small insect pests like aphids, coccids, etc., and thus help in biological control. These are called Predatory mites (Gupta, 1985). Altogether, 557 species of mites known to be associated with plants in India till 1983 were recorded by Gupta. The earliest record of the study of mites in this country was made as far back as 1868 when Peal discovered mites on tea in Assam and named it red-spider (Gupta, 1985).

Mites are not insects but are closely related to spiders. As adults they typically have round or oval bodies with 8 legs. They tend to move quickly over plant surfaces in search of prey. Predatory mites are often slightly larger and a different color from pest mites (Connor, 2009).

4.2.1.2 Habitat and habits

Since most of the plant mites are negatively phototrophic, these are normally found on the under surface of the leaves either in colonies (like members of the families: Tetranychidae, Tenuipalpidae, Eriophyidae) or in solitary forms (like members of the family Tarsonemidae and majority of the predatory mites).But sometimes when the population on the lower surface increases considerably, some of the mites may also occur on the upper surface of the leaves. A large number of mites may be found in the flowers, buds, axils of leaves, etc (Gupta, 1985).

4.2.1.3 Seasonal occurrence

Most of the phytophagous mites and predatory plant mites occur in the field throughout the year in tropical climate except, of course, in rainy seasons when the population declines considerably due to washing away of the leaf population and also during the severe winter months when the egg laying ceases due to dropping down of temperature below the development threshold. Temperature, humidity and light are the important factors influencing the dynamism of mites. Temperature, humidity and light are the important factors influencing the dynamism of mites. photoperiod, temperature and nutrition. Of these, the first two conditions are most important though absence of healthy food may also induce diapausing (Boudreaux, 1963). Diapausing mites or their eggs must undergo a period of diapause development before environmental condition can trigger the resumption of activity. The dispersal in mites is normally accomplished through transportation of hosts as has been seen in case of mites infesting ornamental plants, fruit trees, etc. The dispersal from one field to another takes place through crawling. In some tetranychids, the dispersal is done in very peculiar way called 'Balooning'. When the population of mite in a leaf reaches to a stage that cannot provide enough food to the mite, the dispersal becomes a must. The mites lower themselves from the host plant on a fine silken thread and then subsequently are carried away to a distant place through a gentle wind current. The silken thread acts as baloon or as parachute (Gupta, 1985).

4.2.1.4 Body structure

Unlike insects, with bodies divided into head, thorax, and abdomen, the arachnid body is ancestrally divided into two functional units, the prosoma (the first six body segments) and the opisthosoma (the remaining segments). The body of a mite is further modified in that these original units are fused. A secondary subdivision separates the first two body segments into a structure termed the gnathosoma, specialized for feeding, and the remainder of the body, termed the idiosoma, containing organs of locomotion, digestion, and reproduction.

Most mites show no evidence of external body segmentation, other than the serial appendages. The gnathosoma bears the first two pairs of appendages, the chelicerae, which may retain the ancestral chelate, or pincer-like form, or may be highly modified as stylets for piercing and sucking (Connor, 2009).

4.2.1.5 Life cycles, development and reproduction

Most mites exhibit a fixed developmental pattern, passing through the same number of instars regardless of how much food is available. The most complete pattern consists of egg, prelarva, larva, protonymph, deutonymph, tritonymph, and adult (Connor, 2009).

LIFE STAGES OF PREDATORY MITES

Stage 1: Egg: Oval or oblong, deposited near prey, usually on underside of leaves.

Stage 2: Larvae: 6 legs, usually clear color, may or may not feed.

Stage 3: Protonymph: 8 legs, usuallyvery active and predatory

Stage 4: Deutonymph

Stage 5: Adult, 8 legs, active predator (Gupta, 1985)

Mites exhibit a variety of reproductive strategies and modes of sperm transfer. Ancestrally, mites appear to practice indirect sperm transfer, with males producing and depositing a package of sperm, termed a spermatophore, on the substrate. Females then take the spermatophores into their reproductive tract. This type of reproduction is found in most acariform subgroups, and individuals of the two sexes may or may not be in close contact at the moment of insemination. In known parasitiform groups, males typically use their chelicerae to assist in directly inserting a spermatophore into the female's primary genital opening (as in Ixodida and primitive Gamasida), or the male

chelicerae bear an organ termed the spermatodactyl which is used to transfer sperm from the male's genital opening into secondarily developed sperm induction pores near the bases of the female's legs (Gupta, 1985).

Finally, thelytoky, or all-female parthenogenesis, is found in many groups of mites. Such mites reproduce clonally, with diploid eggs developing directly into females without fertilization (Connor, 2009).

When on leaves, these mites are typically found on the underside near the midrib. Eggs are usually laid along or under the midrib or other large veins, and quiescent forms are most often found there (Kain, D. and Nyrop, J.P., 1995)

4.2.1.6 Ecology of mites

Mites exhibit a breadth of ecological interactions unmatched in any other arthropod group. Mites may be found in all geographic provinces, from tropical rainforests to arctic tundra and rocky outcrops in Antarctica and from desert habitats to the deep ocean trenches. They dominate the microarthropod fauna of the soil where they may be found several meters deep or even in groundwater. They occur in all types of aquatic habitats, including freshwater lakes, streams, seepage areas, and even hot springs. Unlike insects, mites are also quite diverse in marine habitats, ranging from the intertidal zone to the deep trenches (Connor, 2009).

A single square meter of temperate forest soil may contain upward of 250,000 Soil mites, belonging to a hundred different families. In the litter and upper layers of organic soil, mites play many roles in food webs based on decaying plant materials. In such systems, predatory can be found feeding on nematodes, small annelids, collembolans, other mites, and the eggs of insects (Connor, 2009).

However, in terms of the cycling of particular nutrients, notably calcium, mites play an essential role. Mites are also extremely important in the dispersal of bacterial and fungal agents of organic decomposition. Mites feeding on such substrates ingest bacteria or spores that can often pass undigested through the mites guts. The movement of the mites through the soil, with the associated deposition of fecal pellets containing decomposer propagules, provides a much more effi cient dispersal of these organisms than simple physical processes. Female laelapid and macrochelid mites typically attach to insect carriers by grasping host setae or other structures with their chelicerae. Parasitid and digamasellid mites disperse as deutonymphs, often in the space under the

elytra of beetles, and may roam freely over the insect's body. In the Uropodidae, the deutonymph is often specialized for dispersal and may attach to the host by secreting a sticky substance from posterior ventral glands. This material is drawn into a stalk that hardens in air and connects the mite to its host(Connor).

4.2.1.7 Mode of feeding

Not much is known about actual mode of feeding. Among the predatory plant mites, no specificity has been observed though one species may occur only on particular plant while another species may occur on a wide range of plants. The chemical composition of the host plant, leaf size, its texture, vestiture, contents of nitrogen, phosphorus, calcium, magnesium, metal chelates, etc. influence the host specificity of mites. Nitrogen and phosphorus induce mite reproduction while calcium, magnesium and metal chelates retard it (Huffaker *et al.,* 1970). The presence and absence of alternate food in the field influence in making a particular plant more susceptible or resistant to a species (Gupta, 1985).

The members of the order Mesostigmata, Astigmata, Prostigmata show varying types of feeding habits as predaceous, phytophagous, mycetophagous or coprophagous. Some species have more than one type of feeding habits as Phytoseiidae feeds on animal food, nectar, plant juice and pollen (Gupta, 1985).

4.2.1.8 Collection of mites

Hand-picking: Though it is most strenuous, but yet it is profitable as a rich and varied collection of plant mites can be made by this method. The infested leaves or plant parts are examined under a lOX lens in the field and the mites can be picked up by a fine sablehair brush moistened with alcohol (75%) identification of species and, therefore, necessary efforts should be made to get this sex in the collection (Gupta, 1985).

4.2.1.9 Preservation of mites

The plant mites (except Eriophyidae) are best preserved in ethyl alcohol (70-80%). A few drops of glycerol may be added to avoid drying of specimens through evaporation of alcohol (Gupta, 1985).

4.2.2 CHRYSOMYLLID BEETLE

4.2.2.1 Introduction

The Scientific name, Coleoptera, was coined by Aristotle to signify the hardened, shield-like forewings (*coleo*= shield, *ptera*=wing). Beetles comprise 25% of all described animals and plants, single-handedly making them the primary contributor to the world's known biodiversity. The 350,000 described beetle species are members of the largest order of life on Earth, Coleoptera. Many are considered to be serious pests of our homes, forests, crops, and stored products, whereas some benefi cial species are regularly employed as biological control agents (Leibherr, 2013)

4.2.2.2 Body Structure, Life cycle

1. A holometabolous life cycle, wherein the larval stages are developmentally separated from the adult by the pupal stage.

2. Possession of hardened forewings, called elytra, that abut medially. Flight is powered predominantly by the metathoracic wings, which are folded longitudinally and usually transversely to lie under the elytra when the beetle is walking or at rest. The mesothoracic scutellum is usually visible as a triangle situated medially between the bases of the two elytra.

3. A prothorax that is distinct from, and most often freely articulating with, the following mesothorax. The meso- and metathoracic segments are fused to form the pterothorax.

4. A generally depressed body shape, whereby the legs are situated on the ventral surface of the body. The leg bases, or coxae, are recessed into cavities formed by heavily sclerotized thoracic sclerites.

5. Abdominal sternites that are much more heavily sclerotized than the tergites. These sternites may close tightly against the lateral edges of the elytra, protecting the hind body from the attentions of predators and parasitoids.

6. Antennae usually with 11 or fewer segments.

7. Terminal genitalia that are not visible when in repose; that is, the male aedeagus and the female ovipositor are retracted into the abdominal apex when not in use.

Life cycles also can vary in extraordinary ways, depending on the larval food resources used for development. Dramatic variation in reproductive capacity is also observed across the Coleoptera (Leibherr, 2013).

Beetles are among the earliest diversifying groups of the Holometabola. They form a branch on the Tree of Life together with the Neuropteroidea (Megaloptera, Raphidioptera, and Neuroptera). The order Coleoptera is divisible into four major lineages, which are recognized as the suborders Archostemata, Adephaga, Myxophaga, and Polyphaga. Present-day diversity among the four coleopteran suborders is highly skewed toward the Polyphaga (Leibherr, 2013).

Many of the largest families of Polyphaga (e.g., Buprestidae, Scarabaeidae, ***Chrysomelidae***, Cerambycidae, and Curculionidae) include lineages that are intimately associated with angiosperms (Leibherr, 2013).

The *Phytophagus* or Plant-eating Beetle fall into four large families called *Lnriida:* Pulse Beetles, *Chrysomelida* or Leaf eating Beetles, *Cerambycidae* and *Lanziida* (D'abreu, 1915).

The genus Altica is the type genus of the Flea Beetles Alticinae, the largest sub family of the Leaf Beetles Chrysomelidae. It is a widespread genus that is best represented in the Neotropical Region, well represented in the Nearctic Region, with somewhat less in the Palearctic, and a significantly smaller number of species approximately equally represented in the tropics of Africa and the Oriental-Australian Region (Heikertinger and Csiki, 1940). However, Altica is probably poorly known in most areas, except possibly in the Palearctic and to a lesser degree in the Nearctic, because it is taxonomically one of the most difficult genera of Flea Beetles. This is because all species are basically the same color metallic blue-green-bronze with very few reliable external djstinguishing morphological features; therefore, it is almost imperative to examine the male genitalia aedeagus in order to determine the species (Furth, 1980)

Scherer believes that Altica or its ancestors may have originated in Africa before its separation from South America in the Jurassic and, thus, may have entered the New World (Furth, 1980).

4.2.2.3 Host relationship

Most of these host families are perennials and, in fact, many are woody perennial trees. This is rather unusual because most Flea Beetle genera have annual or non-woody perennial hosts. Apparently in several cases, Altica species is able to feed on trees in two or more families, e.g. *A. quercetonun* normally on Quercus (Fagaceae) has been often recorded on Salix (Salicaceae) and *A. tamacis* normally on Tamarix (Tamaricaceae) has been recorded on Salix (Mohr, 1966). Likewise, in North America, *A. ulrni* Woods. Normally on Ulmus (Ulmaceae) has been found feeding on Tilia (Tiliaceae) . Little, if anything, is known about Altica hosts in the Old and New World tropics, but host relationships presumably show some similar trends as in the temperate zones (Mohr, 1966).

There are five primary host families for Holarctic species of Altica: Onagraceae; Rosaceae; Ericaceae; Corylaceae; and Cornaceae. All of these families are hosts for both Palearctic and Nearctic species of Altica. Onagraceae (*Epilobium, Qenothera*) is clearly the dominant host plant family for Altica with Rosaceae (*Rosa, Rubus, Fragaria*, etc.) evidently the second largest. The fact that there are Altica species counterparts, from a variety of host plant families in both the Palearctic and Nearctic Regions Old and New World, may indicate common ancestral lineage. Thus, it is not surprising that in Israel, where there are few of woody perennial species relative to Europe, North America or the moist tropics, there are only a few species of Altica which feed on the two most common Altica-host families - Onagraceae: *Epilobium* and Rosaceae : *Rubus*. Is is worth noting that some species that apparently prefer Onagraceae have also been recorded on Rosaceae, e.g. *A. oleracea* on Strawberry *Fragaria*. It is very important to record host plant information for species of Altica because it may be one of the most useful characters in their determination (Furth, 1980).

4.2.2.4 Life cycle of Altica

The exact life cycle and number of generations are not known for the Altica; In 1974 the most common Israeli Altica, *A. bicarinata*, was reared for one generation from two localities Tel Aviv and Wadi Fatia in Samaria and certain life cycle information was obtained. In both of the above localities the first copulating adults were brought from the field during the first week of March. The orange eggs were laid immediately, singly or in small groups, usually on the underside of the Rubus leaves. The first instar larvae

hatched in approximately 10-12 days and began to feed on both leaf surfaces. The larvae skeletonize the leaves sometimes to The larvae feed for approximately one month and then pupate in the soil beneath the Raspberry bushes; pupation lasts about one week. Therefore, the entire life cycle lasts from 6-7 weeks. It seems evident that there are at least two and probably three generations of *A. bicarinata* per year in Israel. However, the first generation seems to produce the largest populations and sometimes in late April, after the emergence of the adults of the first generation, thousands of beetles can be seen in masses on a relatively small area of Rubus. It is doubtful whether any of the other five species of Israeli Altica have more than one generation per year, but not enough is known yet about their biology (Furth, 1980).

4.2.2.7 Mode of feeding

Adults usually feed on leaves and flowers, while the larvae feed on leaves and flowers (Furth, 1980).

4.2.2.5 Nature of damage

Heavy defoliation may lead to reduced photosynthesis in the plant infested, thus affecting its growth and deveopment. Damage to flowers directly leads to reduced fruit yield (Furth, 1980).

4.2.3 PSYLLIDS

4.2.3.1 Introduction

Psyllids are economically important as pests of several horticultural crops and also as biocontrol agents of weeds. Psyllids (Hemiptera: Psylloidea) are small insects that suck plant juices. The adults resemble miniature cicadas and are sometimes called jumping plantlice. Each kind of psyllid feeds on only one plant species or closely related group of plants. Psyllids are phloem feeding insects which belong to series Sternorryncha of suborder Homoptera. The group comprises over 3000 species in 235 recognised genera, distributed worldwide from the Arctic Circle to the subantarctic islands of New Zealand with its greatest diversity in tropical and south temperate regions (Hollis, 2002). Superfamily Psylloidea comprises 6 families *viz*., Psyllidae, Calophyidae, Phacopteronidae, Homotomidae, Carsidaridae and Triozide. Majority of psyllids are free living and 15 % of known species are gall inducers (Shivankar and Rao, 2010). The collections studied reveal that the Indian fauna is very rich in species of Psyllidae, but

because of their small size and active habits they have been overlooked and our knowledge of their distribution is far from sufficient.(Mathur, 1985)

4.2.3.2 Habit

The insects are phytophagous in both nymphal and adult stages. The adults are generally very active and capable of jumping and flying.(Mathur, 1985)

4.2.3.3 Body structure

Adult psyllids resemble minute cicadas, ranging in length from 1.10 mm. They have two pairs of membraneous wings held roof-like over the body, the forewings usually of a harder constituency than the hindwings.

4.2.3.4 Life cycle

Adult females lay eggs that hatch and develop through about five instars (nymphal growth stages) before maturing into winged adults. Psyllids become abundant in spring when temperatures are warm and host plants produce new growth flushes. Most psyllid species require only a few weeks during the warm weather to complete development from egg to adult. Development and reproduction stop or greatly slow during cool weather, and in some species hot weather can suppress their populations. Most species have about 3 to 5 generations a year, but some species may only have one generation a year. Adults hold their wings rooflike over their bodies and are 1/12 to 1/5 inch long, similar in size to large winged aphids. They have strong jumping legs and short antennae. Nymphs are flattened and less active than adults. Do not confuse psyllid adults with similar looking but harmless psocids honeydew. They breed continuously during the growing season so long as new buds or foliage are available. The nymphal stages are often covered in a small mass of flocculent matter, produced usually from the caudal end of the abdomen.

If liquid excrement with their surface coated with powdered wax and fragments of wax: wax threads, are also present in these rolled leaves. Several kinds of ants, honey bees and wasps frequently visit-these leaves and feed on the honey dew for their food. The nymphs crawl out of the gall when it dehisces or leave the shelter of the roll, and shed their last skin and this moulting is more commonly performed o the surface of an older leaf. Evidently, there are five moults and the earlier shedding of skins is done inside the gall or roll, Adults are translucent white at emergence and their colouration develops fully little after feeding for some time. The renewal of vegetative activity of the host

plant determines the emergence of adult psyllids of the gall-forming species.(Mathur, 1985)

4.3.3.5 Predator of Psyllid bugs

Adult minute pirate bug, *Anthocoris nemoralis*, has been observed feeding on a psyllid nymph (Mathur, 1985)

4.2.3.6 Mode of feeding

Psyllids or jumping plant lice are small phloem feeding insects having piercing and sucking mouth parts. The psyllids have been reported to vector phytoplasma diseases in horticultural plant. A psyllid, *Cacopsylla* sp. (Hemipteridae) infests seabuckthorn (*Hippophae rhamnoides* L.) growing naturally in high altitude regions of north west Himalayas. While feeding, the nymphs and adults of this psyllid insert their mandibular and maxillary stylets into the young succulent leaves, inject saliva and then absorb the liquid food. Excess water with dissolved sugars is subsequently excreted as tiny white droplets of honeydew on to the leaves and stems (Sharma & Sharma, 2017).

The free-living species move about on the host plant and feed on the younger leaves and shoots suitable for inserting their stylets through which the sap is sucked.(Mathur, 1985)

4.2.3.7 Nature of damage

Psyllids suck plant juices and excrete sticky honeydew on which blackish sooty mold grows. Some species secrete pale or white wax masses, pellets or strands, or coverings called lerps. High psyllid populations reduce plant growth or cause terminals to distort, discolor, become galled, or die back. Certain species, such as the olive and tipu psyllids, can cause premature leaf drop. The eugenia and peppertree psyllids cause leaves to develop a pit around the spot where each nymph settles and feeds. Excessive honeydew excretion and wax secretions can also damage plants or property below the infested foliage**.** Copious honeydew secretions by colonies of this psyllid lead to sooty fungal growth. Consequently, the affected leaves and stems get blackened. The uncontrolled infestations often lead to poor plant vigour and reduced berry production. However, despite immense industrial utility, this plant somehow remained grossly unexploited in its natural habitat in north-west Himalayan region. Even berries from the self-perpetuating seabuckthorn stands growing on approximately 12000ha in these high altitude areas are virtually wasted (Sharma & Sharma, 2017).

They also secrete an abundance of a sweet, sticky substance known as honey dew which on falling on the lower branches and leaves causes a disagreeable condition, especially when a black, sooty mould develops and thus seriously injuring the tree. The nymphs of some species cause a distortion and folding of the leaves. One or both margins of the leaves are rolled into the mid-rib on the upper surface and margins gradually become thickened and hard like a gall. The nymphs remain sheltered in these rolled leaves and are often covyred with a copious secretion of white wax (Mathur, 1985)

They cause damage not only by direct feeding but also as vectors of deaseases (Shivankar and Rao, 2010).

4.2.4 APHIDS

4.2.4.1 Introduction

Aphids are small soft-bodied insects, commonly known as plant- lice or greenflies, belonging to order Hemiptera, suborder Stenorrhyncha, superfamily Aphidoidea and family Aphididae (Bhagat, 2012).

Out of 4702 aphid species so far known world over about 1015 species occur in oriental region. Of these, about 653 species belonging to 208 genera represent Indian Aphididae. The food plants of Indian aphids cover 1250 species belonging to 137 plant families, 86 orders and suborders against a total plant species of about 45,000 found in India. Among the areas enjoying subtropical to warm temperate climate in India, diversity and concentration of aphids are more pronounced in northwest and northeast Indian states whereas hotter and drier areas of Indo-gangetic and Peninsular India have less species diversity and poor prevalence.

Aphids are remarkable, evolutionarily exquisite creatures, and are among the most successful insects. Aphid evolution has been shaped through nutrient-driven selection and by the host plants on which they feed, and aphids have responded by developing intricate life cycles and complex polymorphisms. These sap-feeding hemipterans have coped with a hostile world through developing an exceptionally high reproductive rate and passive windborne dispersal, a strategy in which individuals are quite expendable, but survival and prosperity of their genes are guaranteed. Because of their intriguing evolutionary adaptations, aphids were among our most worthy competitors as humans entered the agricultural era (Sorensen, 2012).

They usually live on the young foliage, often causing distortion of the leaves. Some may occur on young twigs or at the bases of stem or may be associated with the roots. Most species of Aphis are attended by ants. About 4,700 species of aphids (Insecta : Homoptera : Aphididae), popularly known as plant-lice or ant-cows, constitute a group of small sized (0.7 to 7.0 mm in length) insects that suck the phloem fluid of plants and about 1000 species are injurious to crops throughout the world.

4.2.4.2 Distribution of aphids

According to Ghosh, review of the literature reveals that out of 27 species dealt with in the paper, 6 are indigenous, 4 palaearctic and 17 either distributed in tropical belt from South-East Asia to Africa or cosmopolitan. Within India, one species is restricted to North-east, 8 to North-west and 6 are found allover the country (Singh *et al*, 2016).

Critical analysis of the collection data reveals that great diversity and abundance of the species of *Aphis* Linn. occurs at higher elevations of India. The biogeographical diversity of the cquntry is well reflected in the distribution, abundance and also in aphid-hostplant relationship. Endemism in Indian species of *Aphis L.* comprises 22.2% as against 55.6% of endemism shown by total Indian Aphididae . However, endemism dominates in northern regions whereas Indogangetic plains are practically without any indigenous species of *Aphis* L. (Sorensen, 2012).

4.2.4.3 Major groups and host affiliations

Aphids, as the superfamily Aphidoidea, belong to the Hemipteran Sternorrhyncha. Aphidoidea has three families: Adelgidae (adelgids), Phylloxeridae (phylloxerids), and Aphididae. Some workers place the Adelgidae and Phylloxeroidea in a separate superfamily, Phylloxeroidea. Adelgids and phylloxerids are primitive " aphids " and older groups, each with about 50 species. They differ from Aphididae by having an ovipositor and reproducing by means of ovipary, whereas Aphididae lack an ovipositor and are parthenogenetically viviparous, bearing live young. Adelgids are restricted to conifers in Pinaceae where some form characteristic galls. Phylloxerids, which may also form galls, occur as the tribe Phylloxerinini on Salicaceae, or as the tribe Phylloxerini on Fagaceae, Juglandaceae, Rosaceae, Ulmaceae, and Vitaceae (Sorensen, 2012). The most unique are paired siphunculi (cornicles) that release an alarm pheromone. These vary from being mere pores on the abdominal surface to being very elongate and sometimes elaborate tubes. Aphids also have a cauda, used to manage honeydew, on the

abdominal terminus. This may vary from rounded, to knobbed, to long and fingerlike. Some aphids produce waxy cuticular excretions and can resemble other Sternorrhyncha.

The taxonomy of Aphididae is quite difficult and subfamily demarcation has been argued through many classifications. Remaudiere and Remaudiere's 1997 classification, followed here, recognizes about 25 aphid subfamilies, with tribal groupings for about 600 genera and 4700 species of aphids (Sorensen, 2012).

4.2.4.4 Life cycles and polymorphism

Thelytokous parthenogenetic viviparity (obligate parthenogenesis where mothers give birth only to daughter young ones), short generation time (about 10 days on average), telescoping of generations (where grandaughters begin developing directly within the daughters which are themselves not yet born) and polymorphism are major traits of aphids that make them highly prolific in reproduction. Many species of aphids display complex life cycles with alternation of sexual and asexual generations and host plant alternation (rajendar singh et al)

The complex life cycles of aphids have caused many specialized morphs to evolve. These have many confusing, synonymous names, but the names are minimized here. Aphid life cycles can be either monoecious or dioecious and involve holocycly or anholocycly.

In the simple and generalized monoecious holocyclic aphid life cycle, a single host plant species is used throughout the year and sexual morphs are produced in the fall, usually in response to decreasing day length. The males and oviparae mate, producing genetically recombinant eggs that overwinter on the host plant and often experience high mortality. In the spring, the fundatrix emerges from the egg, matures, and gives parthenogenetic live birth to nymphs that become viviparae and continue in that reproductive mode through the summer. If the aphid group produces plant galls, the fundatrix is responsible for their production. The viviparae may be apterae (wingless) or alatae (winged), but in some groups all viviparae are alatae. The parthenogenetic reproduction of viviparae allows a very rapid population buildup. At birth, each viviparous nymph has within it the embryos of its daughters and grand daughters, creating a " telescoping " of generations. Apterae, lacking wings and their associated musculature, are optimized for reproduction, and have more offspring per female than do alatae. Alatae invest resources in their flight apparatus and are optimized for

dispersal. Alatae, however, begin progeny production earlier in life than do apterae, giving the alatae relatively reduced number of offspring and a better generational turnaround time. Apterae are produced selectively when nutrient production by their hosts is high. Once an aphid population has increased enough to induce crowding and stress its host's nutrient levels, the population usually switches to alatae production. This allows dispersal to better feeding situations and optimizes the genetic survival of the clone. Alate flight is mostly passive in the wind, and after successfully alighting on their proper host, often by chance, alatae feed for a short time before beginning autolysis of their flight musculature. While precluding further flight, the autolysis self-cannibalistically provides nutrients for their offspring. The production of viviparae continues until fall conditions trigger production of the sexuals (Sorensen, 2012).

A second, more complicated dioecious life cycle has independently evolved among several different aphid groups that show seasonal alternation between differing hosts. This cycle probably evolved in response to the seasonally inadequate supply of nitrogen-based nutrients, especially amino acids, on the primary host. The phloem sap that aphids feed upon has limited nitrogen availability, which inhibits adequate protein synthesis during aphid development. Woody deciduous plants normally translocate amino acids in quantity only during spring foliation and fall leaf senescence. The latter breaks down leaf protein allowing nitrogen translocation to the roots for overwinter storage and future spring plant growth. Aphid groups evolving on, and restricted to, such plants face a nitrogen deficit during summer, when active plant growth ceases and phloem sap is low or devoid of nitrogen.

Other groups, such as Aphidinae, whose ancestors originated on deciduous woody plants, have evolved to leave those primary hosts during the late spring, after the nitrogen flush of foliation has ceased. In doing so, their spring alatae, as emigrants, migrate to herbaceous secondary hosts that actively grow and translocate nitrogen during summer. In fall, however, as these secondary hosts die back, the aphids return to their woody primary host by producing winged migrating males and gynoparae, the latter giving rise to oviparae. Upon returning, the aphid's sexuals (its males and oviparae) capture the primary host's fall nitrogen flush and mate to lay their overwintering eggs in anticipation of the spring nitrogen flush (John T. Sorensen).

4.2.4.5 Aphid behavior

Evolutionary selection has dictated efficiency in aphid behaviors as well as the expendability of individuals. To feed proficiently, aphids insert and ratchet their rostrum-borne stylets between plant cells, seldom penetrating any until reaching the phloem sieve tubes to extract sap. The stylets are lubricated by pectinase-containing saliva that both loosens plant cell bonding and forms a stylet sheath that is left behind when the stylets are withdrawn. To cope with a sap diet, aphid guts have specialized groups of cells, mycetomes, containing rickettsialike symbiotic bacteria, mycetocytes, which aid in synthesis of nutrients. These bacteria are passed between aphid generations and have coevolved with, and differentiated between, aphid phyletic lineages (Sorensen, 2012).

Morph-specific behaviors promote genetic survival of the individual and its clones. Alatae initially taking flight are attracted to the short wavelengths that predominate in the sky, which they fly toward to optimize dispersion. During descent their preference changes to the longer light wavelengths reflected by plants, especially the yellow hue of senescent plants that are better nitrogen sources. After alighting, they accept a plant for feeding only after briefly probing their rostral tip below the plant's epidermis to sense the presence of specialized secondary plant compounds that are of no nutritional value, but are specific to their given host. Apterae, in contrast, move only when necessary to procure a better feeding site or avoid predation (Sorensen, 2012).

Ants may tend some aphid groups in a form of facultative mutualism in which the ants may actively "farm" their aphid "cattle" moving them among locations. In exchange for the aphid's sugary honeydew, the ants protect them from predation and parasitism (Sorensen, 2012).

4.2.4.6 Importance of aphids

Aphid damage is among the most serious of agricultural and horticultural problems. A pest aphid species may affect only a very specific crop, a group of related crop hosts (e.g., crucifers), or may be quite polyphagous within and between plant families. Many of the notoriously polyphagous aphid pests represent sibling species complexes that are morphologically identical but differ in karyotype. Generally, such aphid pests comprise anholocyclic clones, or biotypes, that differ in host preferences, the ability to transmit diseases, or resistance to pesticides (Sorensen, 2012).

4.2.4.7 Nature of damage

Aphids cause damage and lower yields in several ways. They can build to high population densities, removing plant nutrients, and may damage plants by removing enough sap to cause withering and death. If not washed off, aphid honeydew excrement can build enough on plants to be a growth medium for sooty molds that impair photosynthesis and promote other fungal diseases. Salivary secretionsof some aphids are phytotoxic, causing stunting, leaf deformation, andgall formation, which is of particular concern to horticulture. Even if otherwise asymptomatic, aphid-feeding effects may affect plant hormone balances changing host metabolism to their advantage and essentially hijacking the plant's physiological functions. The most serious problem posed by aphids is the vectoring of plant viruses. Virus-infected plants often show an aphid-attractive yellowing and have increased free amino acids, so aphids benefit by virus transmission (Sorensen, 2012).

Aphids attached to other plant parts such as stems or twigs may cause stunted growth, early leaf fall, or twig mortality, but aphid damage very rarely kills the plant.

4.2.5 HEARTWOOD BORER

The larva recorded was white in colour with a circular abdominal rings and a black mouth region. It was about 11-12 mm in length. The damage was severe compared to the size of the larvae. The legs were not developed. The time of collection of larvae was late March. The shape of the cavity was highly irregular. Frass was powder like in form. Only one case of boring was recorded. Whole of the woody portion including the heart wood was tunneled, except for the bark and perpherial portion of the wood (Paine, 2009).

4.2.5.1 Introduction

Insects that are borers belong to a wide range of taxonomic groups, but they all share a common life history trait: They spend all or part of their larval life feeding within the tissues of their host plant. Some borer species deposit eggs within host plant tissues, whereas other species oviposit on the external surface and the larvae bore into the plant. Although there may be some feeding activity within the phloem and cambial tissues, the larvae typically excavate feeding galleries within the woody tissues of perennial plants, within the stems of annual plants, and within the stalks or stems of grasses. Adult borers are free-living outside the host plant. Insect orders that include species commonly

referred to as borers are Lepidoptera, Coleoptera, Hymenoptera, and Diptera (Paine, 2009).

4.2.5.2 Feeding strategies

Larval borers may feed within other plant tissues, including roots, stems and twigs, meristems, fruits, conductive tissues, galls, and bark. The variety of plant tissues that are used by borers also spans an array of plant groups that range from the ferns and gymnosperms to the grasses and dicotyledonous angiosperms. All plant tissues may be subject to borer colonization. Larvae of a number of families (e.g., Cerambycidae and Hepialidae) may construct feeding tunnels, or galleries, within the large roots of broadleaf trees and conifers, which may weaken the trees directly or provide entry points for invasion by pathogenic fungi (Paine, 2009).

At the other extreme, there are many species of insects that colonize the meristematic tissues at branch terminals, tips, twigs, and canes. Some of these insects feed in the phloem tissues girdling the twigs, whereas larvae of other species burrow through the growing tips and into the elongating stems. These types of larval feeding can reduce plant growth, apical dominance, and plant form. Between the twigs or apical tips and the roots is the main stem or trunk of the tree. The woody xylem tissues, cambial layers, phloem tissues, and bark may all have different groups of specialist borers (Paine, 2009).

In different feeding strategies, bark beetle larvae feed within the cambial and phloem tissues of their hosts, whereas larvae of many species of longhorned and fl atheaded borers feed in the outer layers of phloem and cambium, but then bore deep into the wood to pupate. Alternatively, many other cerambycid and buprestid species feed almost entirely within the wood of their host trees. Larvae of cossid moths also feed entirely within woody tissues and may take several years to complete their larval development. Woody plant tissues are not as rich in nutrients as the cambial tissues, and the quality deteriorates as the tissues age. Consequently, many wood borers may have prolonged larval development and long generation times (Paine, 2009).

Larvae in all these families can bore through freshly dead or dying wood by using their well-developed, anteriorly directed mandibles. Laterally expanded thoracic segments or abdominal ampullae serve to anchor these larvae in their tunnels, facilitating purchase by the mandibles on the wood surface. Leg reduction has proceeded during

diversification of cerambycid borers, with larvae of more basally divergent subfamilies such as the Prioninae and Lepturinae having shortened thoracic legs, whereas larvae of the highly derived subfamily Lamiinae are legless (Paine, 2009).

4.2.5.3 Recorded stem borer in Hippophae

Asian Long Horned Beetle was recorded from Hippophae tree. In its native range the primary hosts of *Anoplophora glabripennis* are poplars (*Populus* spp.), maples (*Acer* spp.), elms (*Ulmus* spp.) and willows (*Salix* spp.), but it also attacks linden (*Tilia* spp.), Russian olive (*Elaeagnus angustifolia*), birch (*Betula* spp.), horsechestnut (*Aesculus* spp.), sea-buckthorns (*Hippophae* spp.), London plane tree (*Platanus* spp.),and panicled golden-rain tree (*Koelreuteria paniculata*). Outside its Asian native range (i.e in USA, Canada and Europe) the host species thus far found attacked by *A. glabripennis* include maple, willow, elm, birch, horsechestnut, London plane tree, linden, mountain ash (*Sorbus* spp.), poplar, hornbeam (*Carpinus* spp.), beech (*Fagus* spp.) and plum (*Prunus* spp.) (Smith & Jinquan, 2009).

4.2.5.4 Life cycle and behaviour

The beetle completes most of its life cycle inside its host tree. Adult beetles emerge through exit holes from late May through October. However, peak abundance typically occurs from late June through late July, but may extend into early September in colder climates. Adult beetles feed on the outer layer of twigs and the petiole of leaves, and the primary leaf veins. Newly eclosed female beetles require 9–15 days, during which they feed, before becoming sexually mature. The adult females then chew small pits, referred to as oviposition pits, sites or scars, into the bark surface into which they inject a single egg per pit (Smith & Jinquan, 2009).

Upon hatching young larvae tunnel under the bark for about three weeks, slowly destroying the vascular system and disrupting sap flow of infested trees. Older larvae then tunnel into the heartwood where their feeding slowly destroys the structural integrity of infested trees. During the same year that eggs were laid, both young and older larvae may be found feeding within infested trees from June to November (until first frost). ALB adults remain for up to seven days before chewing an exit hole and emerging. Development from egg to adult requires approximately 12–24 months. Repeated attack of the same tree over several years leads to dieback of the crown, structural deterioration and, eventually, death of the tree.Frass (wood shavings and

sawdust) produced by larval feeding and tunnelling. Frass can be found on the bark surface (i.e. crevasses and branch crotches) and on the ground underneath the tree, particularly from May to September (Smith & Jinquan, 2009).

4.2.6 GROUND BEETLE

The beetle was found to be very small i.e less than 3mm in length and was having a circular body with a metallic dark blue elytras. The role these beetles are not known but interestignly were found as 'residents' in the nodule masses of Frankia infected roots of large trees of Hippophae, both male and female. The role of these beetles is not observed, but provides scope for in depth underground ecosystems associated with the Hippophae root system.

When the wildling of Hippophae, along with the ground beetles, was bought with the entire rhizospheric soil in late March and potted in a container, the wildling survived. With that it was observed that with the first showers of monsoon in mid June the Ground Beetles were coming above the soil and took off from the branches of the wildling with a wavy flight in most cases.

4.2.6.1 Introduction

The family Scarabaeidae is one of the interesting and largest family of Coleoptera, which contains more' than 30,000 species throughout the world.

The most striking cases are known from high altitude ground beetles: The distributional areas of primary wingless Carabidae species are commonly restricted to single slopes or valleys, whereas the most closely related occur in directly adjacent areas of the mountains. Morphology-based analyses in numerous Himalayan arthropod taxa indicate extremely limited distributional ranges of these species and the occurrence of strictly allopatric speciation. Various Himalayan endemic ground beetle species groups were identified showing no close relationships to lineages occurring in areas adjacent to the Himalaya. They inhabit humid soils and leaf litter of mountain cloud forests or alpine meadows, and they hide under large stones, rotten trunks or in humid crevices during the day. These species are strictly adapted to temperate or even alpine environments and to relatively humid habitat conditions (Schimdt, 2012).

The habitat preference characteristics and winglessness of these beetles both seem to strongly limit their distributional ability (Schimdt, 2012).

4.2.7 PLANT HOPPERS

The plant hoppers of all the life cycles including male and female was seen severely infesting the *Hippophae salicifolia*. They produced wax-like wavy threads hanging from the tip of their abdomen. The infested medium sized trees were those without a well-developed thick bark. The thickness of the twigs of the infested trees, were very smaller compared to that of a healthy female tree in the nearby region.

4.2.7.1 Introduction

Aphids are generally recognized as the most important plant virus vectors. Leaf hoppers and planthoppers are second in importance. Most of the hoppers multiply in plants (Pasalu, 2014).

4.2.7.2 Life cycle of Plant Hopper

Females lay 300-350 eggs and macropterous females lay less egg. The eggs are thrust in a straight line generally along the mid-region of the leaf sheath, though sometimes eggs are laid on the leaf mid-ribs. Eggs are covered by the dome shaped egg plug secreted by the female. Only the tip of the egg protrudes from the plant surface. The egg laying sites appear as brownish streaks. Eggs hatch in about 6-9 days. The newly hatched nymph is cottony white and turns purple brown within an hour. It feeds on the plant sap and undergoes five moults to become an adult. It takes 10-18 days to become an adult as the process depends on the availability of the food, density during development and other environmental factors. Depending on the length of the crop the number of generations varies. Adult emergence which begins at dawn and continues for 4-5 h takes place at the basal part of the host plant. Adult white backed planthoppers are dimorphic. Macropterous males and females and brachypterous females are very common. The adults prefer to stay on the upper portions of the stem. The pre-oviposition period ranges from 3-8 days. A fecundity of 164 eggs has been reported. The eggs hatch in 6 days. Nymphs are white to strongly mottled dark grey or black and white in colour. They undergo five moults and reach adult hood in 12-17 days (Pasalu, 2014).

4.2.7.3 Nature of damage

In the initial stages of infestation round yellow patches on the plant are seen which soon turns brownish due to drying up of the plants (Pasalu, 2014).

4.3 Nursery pests causing damage to the growth of Hippophae seedlings are

4.3.1 Caterpillar ‘C1’

Two morphs of caterpillars were found. One mimicing the green twig, while the other cater pillar mimiced the dry twig. The shoots were attacked by the caterpillars. The leaves turned yellow with small dark brown spots. The leaves appeared to be wrinkled the day after the infestation and seen to be drying out after 4-7 days of infestation. These obviously affected the growth of the seedlings even though death was not caused. The mimicry was so astonishing that the caterpillars were very hard to identify even after photographing them.

4.3.1.1 Introduction

The larvae of butterflies, skippers, and moths of the order Lepidoptera are generally known as caterpillars. Caterpillars come in a diversity of sizes, shapes, and colors. The most common form has a conspicuous head, a thorax with three pairs of legs, and an abdomen with five pairs of prolegs that bear crochets (hooks) that enable the caterpillar to cling tightly to or wedge itself between materials (Stehr, 2009).

Nearly all species of plants are fed upon by caterpillars, and many caterpillars are important pests. They also form galls; are scavengers on dead plant materials, fungi, and animal materials such as f eathers, wool, or fur (clothes moths, Tineidae); and some are important pests of stored products (meal moths, Pyralidae) (Stehr, 2009).

4.3.1.2 Biology and ecology

Caterpillars are commonly encountered because many are leaffeeders and are not concealed feeders, although they may be cryptically shaped or colored. The realistic twig mimicry and behavior found in some inchworms (Geometridae) are complete with body markings that resemble bark irregularities, scars, and stipules.

Most caterpillars move by a wavelike movement of the legs from rear to front. However, those with reduced numbers of prolegs or none at all proceed in a different manner. Caterpillars may be aposematically colored to advertise that they are distasteful or poisonous. They may also bear diverse lobes, spines, horns, knobs, and urticating hairs or spines that may or may not be irritating in some way. Most caterpillars that feed in protected or concealed locations do not possess such structures or defenses (Stehr, 2009).

4.3.3 CHRYSOMYLLID BEETLE

Not many individuals were recorded. Only one incidence of feeding on leaves was recorded. Would have been introduced along with the vegetative material or would have been present on some other host plant in the Ranichauri region.

4.4.4 BORER

Only one cutting was found with a boring larva. Would have been introduced to nursery in Ranichauri along with the vegetative material.

Result in gist:

Out of 3 replications of each infected part i.e Cambium, Tap root and Primary root used for isolation of pathogen, 3 different fungal pure cultures were obtained. During lab studies, the growth of the fungal mass was very slow characterized by non sporolation in all the three pure cultures. This could be possibly because that the temperature range of BOD i.e 18-24^0 C was supposedly higher than the temperature of Yamunotri. Hence could have favoured healthy growth of hyphal mass, as sporolation is carried out during unfavourable external conditions. Dew drop like secretions was very conspicuous in Cambial fungus and R2 (Root fungus). Absent in R1Pathogenecity test could not carried out to confirm the isolated fungal specimens as Pathogens.

Altogether 6 species of Insects, a species of Predatory mites and a very small species of Ground beetle were found from the collected samples. 8 Entomological specimens in whole.

Out of 288 cuttings planted, only 28 cuttings were found to have survived with a developed root system at the end of 3 months. Survival percentage during 1st, 2nd, 3rd reading and end of 4 months of was found to be 49.65%, 20.48%, 9.72% and 4.51% respectively.

The highest survival percentage during 4th reading was observed to be 19.44 % in Rooting media R1 (Sand) and the lowest rooting percentage was observed to be 0 % in R3 (FYM), R5(Mother soil) and R8 (FYM). But the results should be quantified on large scale propagation.The highest rooting percentage during 3rd reading was observed to be 12.5% in T2 (250 ppm) followed by 8.33% in T1 (100 ppm) and T3 (400 ppm).

CHAPTER-5

SUMMARY AND CONCLUSION

CHAPTER 5 - SUMMARY AND CONCLUSION

The present investigation entitled **Effect of Entomological and pathological agents on the vegetative propagation, growth and yield of Natural and Nursery population of *Hippophae salicifolia* D.Don.** covers the following aspects.

1. Identification of pathogens affecting the vegetative propagation of *Hippophae salicifolia* D.Don semi-hard wood cuttings in Ranichauri Forest nursery.
2. Identification of insect pests/mites/natural enemies of pests on *H. salicifolia* in Yamunotri provenance of Uttarakhand.

The experiments were carried out during the year 2016-2017 in the nursery of the department of Forestry and lab of Pathology Department, College of Forestry, V.C.S.G. Uttarakhand University of Horticulture and Forestry, Ranichauri.

Two emerging factors that are altering interactions among insects and forests are the increased movement of exotic insect species into novel geographic areas, and the changes that are becoming evident as a result of global climate change. Exotic species have the potential to alter the species composition of forests (Arya, 2016).

Damage by this insect in its native range is very limited due in part to host resistance. The movement and impacts of exotic species pose a major threat to forest habitats around the globe. Increased trade results in the movement of both trade items and packing material that all too often harbors unknown insects and other organisms.

Yamunotri region is highly susceptible to this because of increasing global tourism and rapid urbanisation in the form of construction of Hotels, Restaurants etc.

Other insect outbreaks may be a result of changes in the global climate. Elevated winter temperatures are thought to have contributed to the spread to the north of the mountain pine beetle, *Dendroctonus ponderosae*, in British Columbia, Canada where it has caused mortality of pines, especially lodgepole pine, over millions of hectares. Previous outbreaks had apparently never moved this far north, perhaps due to limitations on the insect posed by cold winter temperatures. As the global climate continues to warm, we can expect to see additional effects on insect populations and their interactions with forested areas.

Approximately half of the insect orders are directly or indirectly associated with trees. As with humans, insects use trees for food, shelter, support, and travel. Among all

plants, trees present the most diverse habitats for insects to occupy. Insects feed on all parts of the tree, that is, vegetative structures such as leaves, stems, and roots and reproductive structures such as flowers, fruits, and seeds. Some insects are specialized to feed on phloem and/or xylem tissues, dead sapwood, and heartwood. Insects that feed on these structures and tissues vary in size from 1 – 2 mm (scale insects) to 6 cm (longhorned beetles). Life cycles (from egg to adult) can be completed in a few days or weeks (aphids) or be prolonged for 50 years (metallic wood borers).

Insects affect the growth rate and mortality of trees by feeding on various parts of the tree. Thus they affect the life cycle of trees by influencing the size of the seed bank, the amount of recruitment, the growth of individuals in height and volume, and the reproduction and dispersal of the tree species. Insects consume seeds within fruiting structures or on the ground, thus reducing the size of the seed bank. They consume young seedlings, thus decreasing the recruitment of new trees into the population. They kill tips and shoots, suck plant fluids from the phloem and xylem, and consume the foliage, thus reducing the photosynthetic capacity of the tree and, as a result, reduce the growth of individuals. Insects also kill trees by reducing their growth rate so they cannot compete with other individuals of the same or different species for light, space, water, and nutrients.

The atmospheric concentration of CO_2 has increased from a preindustrial level of about 270 ppm to a current level of about 381 ppm, an increase of over 100 ppm or 41%. Many of the changes in insect populations likely to result from elevated CO_2 will be brought about by changes in plant chemistry (Dudley, 2002).

The chemical changes in plants result from increases in plant carbon, decreases in nitrogen, and increases in levels of defensive compounds such as phenolics. In addition, global warming, which will result from elevated levels of greenhouse gases, may increase the reproductive capabilities of some insects and change their distributional ranges. This could change the abundance of some pest species and disease vectors.

Studies on the green peach aphid, *Myzus persicae* , a pest of many crops, suggest that elevated temperature increases aphid population growth rate and thus the likelihood that aphids will become more important pests in the future. In this case, both elevated CO_2 and elevated temperature increased aphid densities in experiments.

Greenhouse gases are likely to change insect distribution patterns both directly, via increases in temperature and rainfall, and indirectly, via changes in the distribution of host plants. Research on a sample of 35 non-migratory European butterflies showed that 63% had ranges that shifted to the north by 35 – 240 km during the 20th century, while only 3% shifted to the south. Thus for many insects, global warming has already changed range boundaries. The data appear to be robust because for most of these species, northward shifts have been shown in more than one country.

The northward shifts of the butterflies are of the same magnitude as the shift in climatic isotherms, which have moved about 120 km north as Europe has warmed by about 0.8°C. Since then, northerly range shifts have been shown for European grasshoppers, longhorn beetles, lacewings, carabid beetles, aquatic insects, millipedes, woodlice, spiders, soldier beetles, and dragonflies.

Invasive pests that gained entry into this cold arid region of India have cost catastrophic damage on commercial agriculture, horticulture crops and forest trees. Arya and Joshi (2016) have reported 20 species of Coleopteran insects from Nanda Devi Biosphere Reserve, Western Himalayas, Uttarakhand, India. Beetles were sampled over two years of study in different elevational zones of Binsar Wildlife Sanctuary in district Almora, Uttarakhand, India. A total of 734 individuals of beetles, representing 23 species, 18 genera and 6 families, were recorded during the study period. Beetles community showed a significant variation along different elevational zones as abundance and species diversity of beetles declined with increasing altitude across different sites. Climatic variables significantly influenced the beetle community as higher species richness, abundance and diversity were recorded in rainy season followed by summer and winter, respectively (Arya, 2016).

Furthermore, little is known about the reasons underlying the remarkably high diversity, mechanisms of speciation, or origin of the Himalayan insect fauna (Arya, 2016).

The Indian Himalaya has been identified as one of the most sensitive landscapes and biodiversity hotspots undergoing unprecedented changes due to climate change and global warming. Changes in the mean climate and climate variability as well as extreme weather events are the direct effects of climate change, whereas changes in water

availability and biological organisms are the indirect effects of climate change (Kubitzki, 2016).

Mountain areas of Yamunotri regions are characterized by inaccessibility, fragility, marginality, diversity, niches and adaptation mechanisms.Many economically important diseases of crops which are caused by viruses and mycoplasma like organisms are spread by insect vectors, particularly aphids, leaf and plant hoppers, thrips and whiteflies. Some of these small insects can undertake windborne migrations over ranges of tens or even hundreds of kilometres. They pose threat to the Population of the Hippophae. During the daytime, airborne insects can be carried upto altitudes of hundreds of metres by convection motion of the atmosphere (Dudley, 2002).

Comparison of the results from the Phillipines and China suggests that short range migration tends to predominate in tropics, while longer-range movement is the norm in temperate regions (Dudley, 2002).

It is interesting to note that out of 653 species of aphid known so far from India sexual forms of 161 species/subspecies (24.6% of Indian aphids) are known and the rest are recognised by parthenogenetic (asexual) morphs. Out of sexuales of 161 species/subspecies, 153 species/subspecies are distributed in the temperate regions and only 8 are so far known to produce sexuales in the tropical regions of India. the distribution of aphids producing sexuales is substantially higher in northern region (altitudinal areas) and much lower in the plains. Most of the sexuales are known to occur at altitudes varying between *ca*. 750 m and *ca*. 4500 m during colder part of the year when day length is short and temperature is low (Riley, 1995). Thus such a high diversity pose a serious threat if the global mean temperature keeps rising by time.

Altogether, 6 species of Insects and a species of Predatory mites were found. The list seems too small but serves as alarm to the temperate Himalayan fauna directly associated with the change in climatic patterns across the Himalayas.The fungal pathogens isolated from the lab were sent to ITTC. The Indian Type Culture Collection (ITCC) was established in 1936 with a view to furnish the knowledge on living fungi. It is the largest fungal genetic resource centre in India. ITCC is an affiliate member of the World Federation for Culture Collections (WFCC) and is registered with the World Data Centre for Microorganisms (WDCM, registration number 430).

The insects and mites specimens associated with *Hippophae salicifolia* were collected and sent to Entomology division, GKVK, University of Agricultural Sciences, Bengaluru, for the taxonomic level identification.

- Only about 4.51% of the 288 cuttings survived as the healthy five month old seedlings. Even though the whole cause for the failure of cuttings is not the soil borne fungal pathogens alone, it is sure that these cause mortality of cuttings in combination with other physiological and genetical aspects of *Hippophae salicifolia* plant.
- The variation of rooting media (8 substrates) or the rooting hormone concentrations (3 treatments) does not possibly provide any advantage for the Hippophae cuttings for healthy and infection free rooting & growth, if the cuttings are infected by fungal agents from the initial stages of vegetative propagation.

Recommendations:

- Considering the present ecological and possible future economic potential of the *Hippophae salicifolia* plant in Uttarakhand, more comprehensive and advanced scientific study in In-situ is needed to assess the vulnerability of this entire ecological system than the Ex-situ.
- Creating the data base of pests and pathogens associated with Sea buckthorn, by studying their complete life cycle would provide solutions to formulate successful projects for the development and conservation of the plant populations

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PLATE 1: Frankia Collection

Selection of mother tree

Inspection and digging of roots

Young nodules of Frankia

Older nodules of Frankia

Relative size and length of Frankia

Frankia nodule masses

PLATE 2: Isolation of Soil borne fungal pathogens

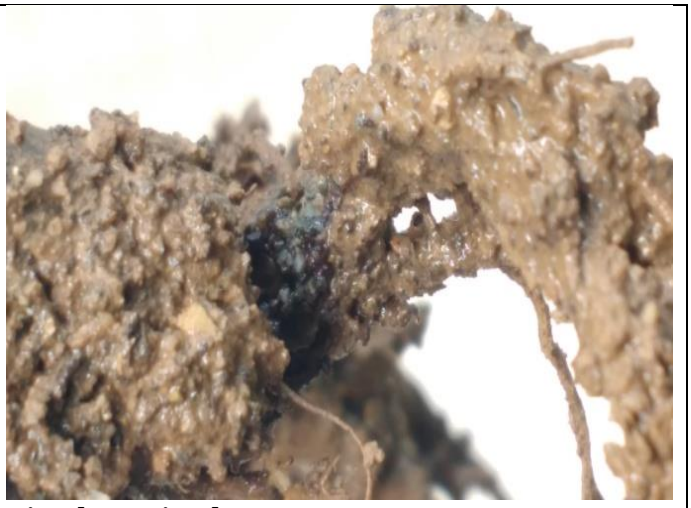
 **Pathogen infected cuttings**	 **Discolouration by Root rot**
 **Cuttings failed to root**	 **Yellowing of leaves**
 **A single rootlet survived from fungal disease**	 **Rooting surface with rot symptoms**

Plate 3: Vegetative propagation

Collection of cuttings on March 28th, 2017

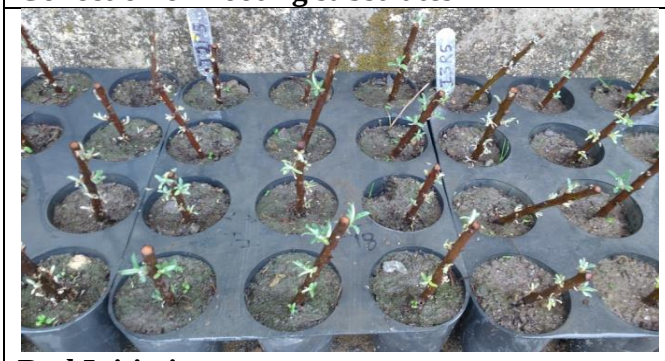
Ideal cutting of pencil size and 15 cm length

Female(L) and male (R)Cuttings

IBA treatment to cuttings for 36 hours

Collection of Rooting substrates

Rooting containers



Bud Initiation

Three month cuttings

Plate 4: *Hippophae salicifolia* plant

Hippophae fruit

Hippophae bark (old tree)

Hippophae shoot

Hippophae wood cross section

Hippophae tree

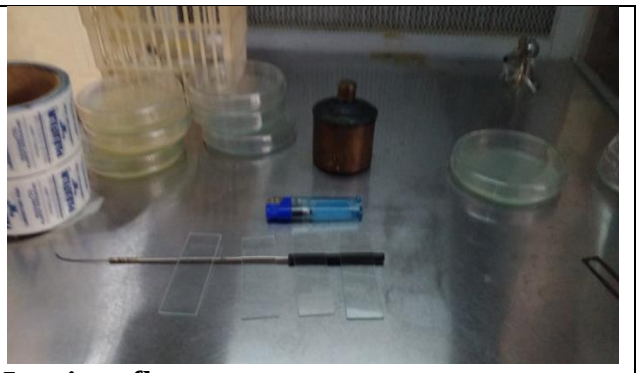
Hippophae bud stage

Hippophae root system

Frankia in Hippophae root

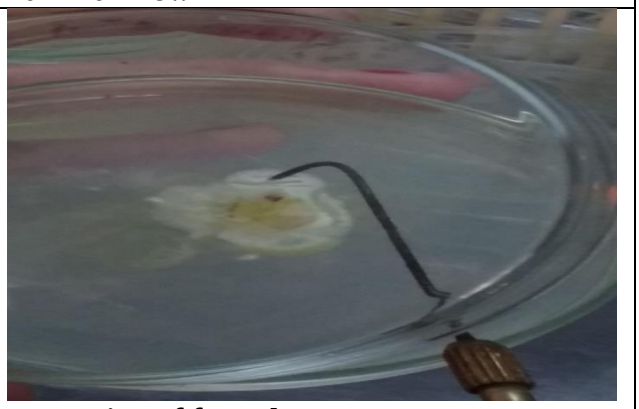
Plate 5: Lacto Phenol Blue staining technique

Lacto Phenol Cotton Blue



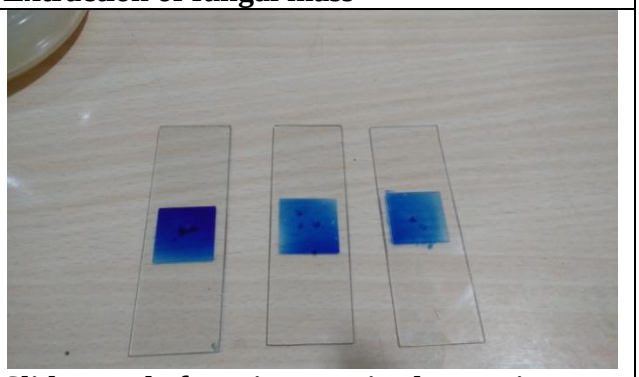
Laminar flow

Fungus to be stained

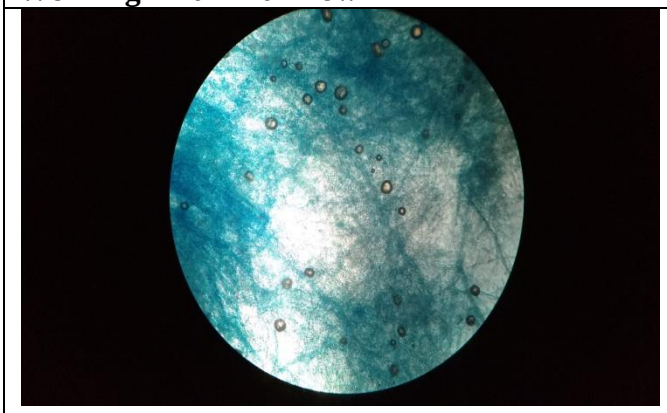


Extraction of fungal mass

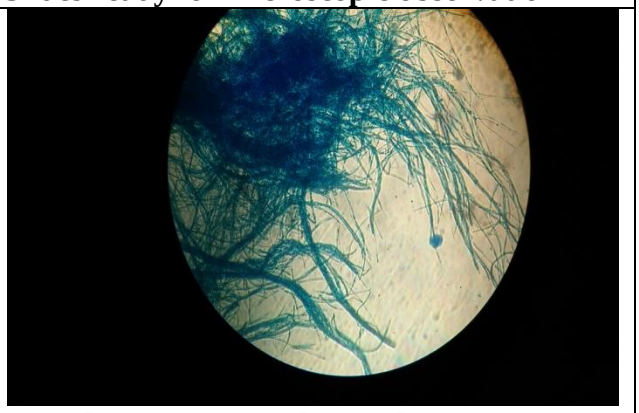
Working in laminar flow



Slides ready for microscopic observation



Microscopic picture of Root fungus



Sporolation was not observed

Plate 6: Pests of *Hippophae salicifolia*

Wood boring larvae. Irregular tunnels are also seen.

Relative size of the wood boring larva. Around 11-12mm.

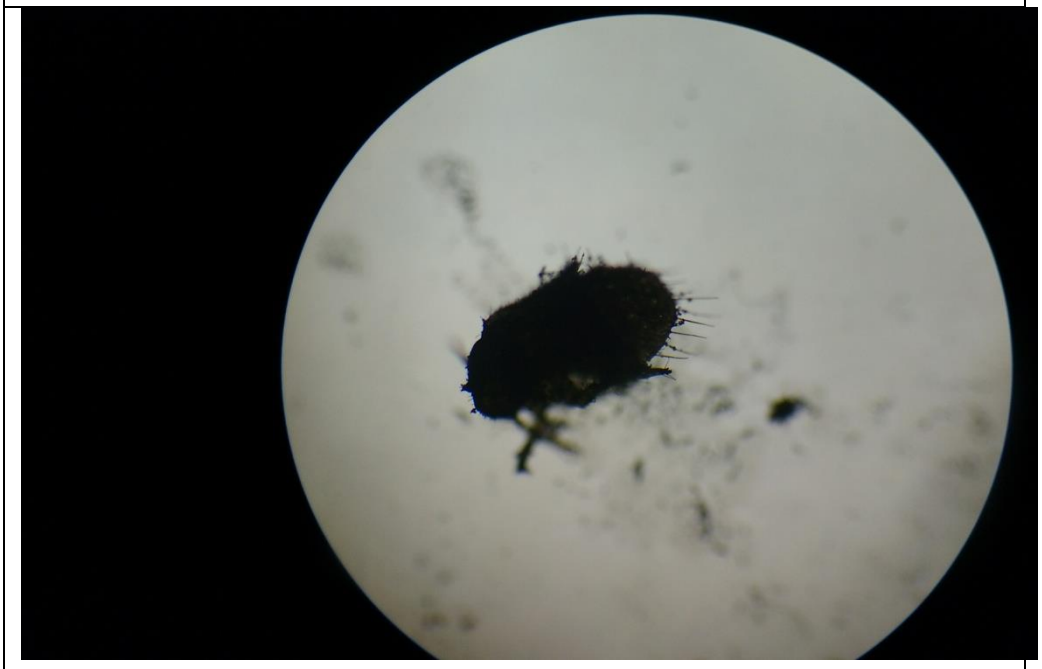
Caterpillar: Brown morph.

Caterpillar: Green morph.

Shoots damaged by caterpillar. Drying is clearly seen

Microscopic view of the Predatory Mite.

Microscopic view of Plant hopper Nymph



Microscopic view of Adult Aphid

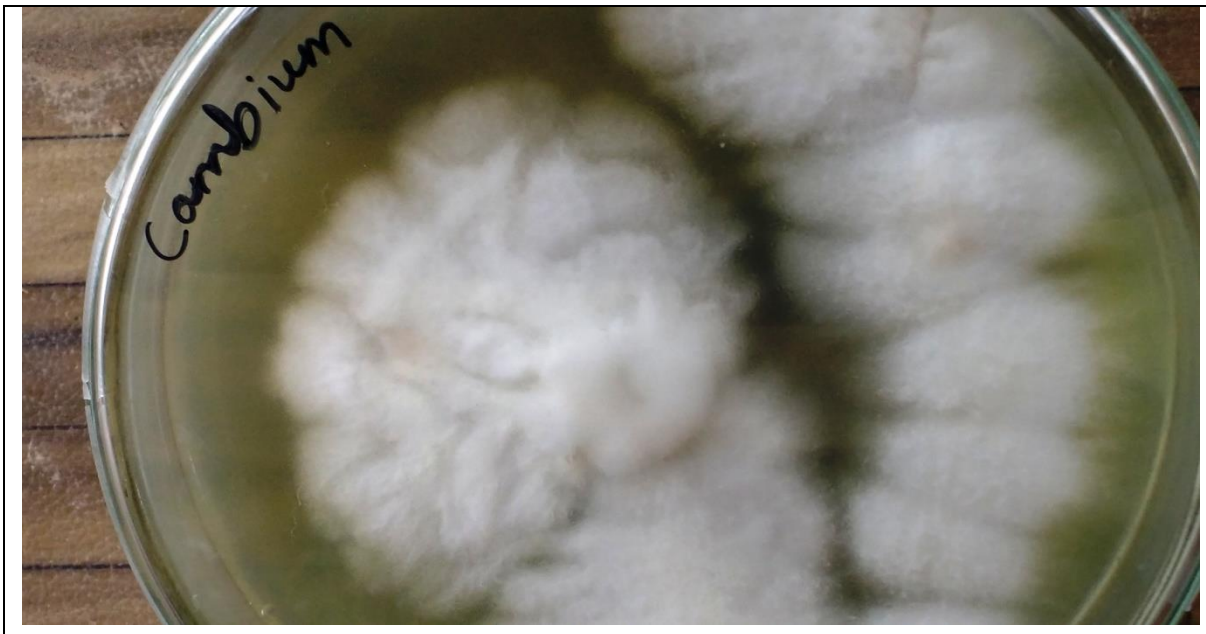
Dense colonies of Aphids on Hippophae male plant

Congregation of plan hopper, Red-coloured mite and aphid

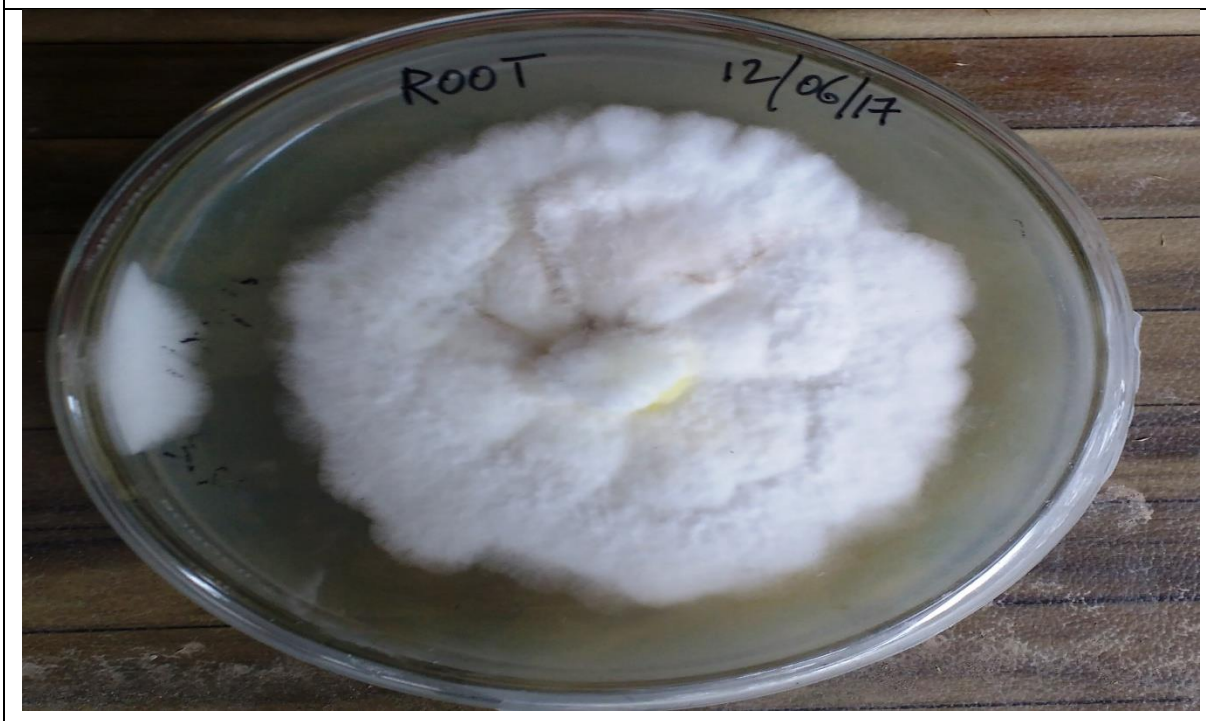
Altica beetle on Hippophae plant

Waxy thread like secretions by Plant hopper.

Plate 7: Isolation of fungus from root and cambium



Fungus isolated from Cambium tissues at the collar region (C1)



Fungus Isolated from Root (R1)

Fungal specimen isolated from (R2): Dew drop like secretions can be seen

Preparation of test tube slants for inoculation of pure culture of isolated fungus

Fungal specimens cultured in slants to send to ITTC, New Delhi, for identification

Table 1: Results of Vegetative propagation trials w.r.t effect of soil borne fungal agents on *Hippophae salicifolia* cuttings

T_1 =Treatment 1 (100 ppm of IBA) T_2 = Treatment 2 (250 ppm of IBA),

Sl. No.	Rooting Medium (Planted on 30-03-2016)	1st reading after one month (Bud initiation) 01-05-16		2nd reading after two months (Bud initiation) 01-06-17		3rd reading after three months (Bud initiation and rooting) 02-07-17		Plants surviving after 4 months 01-08-17
			Total		Total		Total	
1	T1R1	12	33	08	18	04	11	07
2	T2R1	12		06		05		
3	T3R1	09		04		02		
4	T1R2	10	24	02	07	00	03	01
5	T2R2	08		03		02		
6	T3R2	06		02		01		
7	T1R3	06	25	04	09	02	02	00
8	T2R3	08		03		00		
9	T3R3	11		02		00		
10	T1R4	07	13	02	04	01	02	01
11	T2R4	02		01		00		
12	T3R4	04		01		01		
13	T1R5	04	13	01	03	00	00	00
14	T2R5	04		01		00		
15	T3R5	05		01		00		
16	T1R6	01	07	00	03	00	02	01
17	T2R6	06		03		02		
18	T3R6	00		00		00		
19	T1R7	02	12	01	10	01	08	03
20	T2R7	04		04		03		
21	T3R7	06		05		04		
22	T1R8	05	16	01	05	00	00	00
23	T2R8	06		02		00		
	T3R8	05		02		00		
			143		**59**		**28**	**13**

T_3 = Treatment 3(400 ppm of IBA) R- Rooting Substrate

Table 2: Insects/ Mites associated with *Hippophae salicifolia* in Yamunothri

Sl. No.	Insect (I) & Mite (M)	Social habit	Location on plant	Nature of damage	Incidence
1	Altica Beetle (I)	Usually solitary	Vegetal buds	Do not know	Not frequent
2	Plant Hopper (I)	In dense colony	Branches of young shoots	Reduce thickness of twigs, drying of buds.	Very often
3	Aphids (I)	In dense colony	Branches of young shoots and buds	Reduce thickness of twigs, drying of buds.	Very often
4	Ground Beetle (I)	In group	Nodules of the Frankia	Do not know	Only on two occasion
5	Heart wood Borer (I)	Solitary	Heart wood	Reduced tree growth, Disintegration of heart wood	Recorded only once.
6	Caterpillar (I)	Usually one or two (As in my case)	Camouflaged between leaves	Drying of shoot and wrinkling of leaves	Not so frequent
7	Psyllid Bug	Sparse group	Twigs	Do not know	Not so frequent
8	Predatory Mites (M)	Solitary	All over the tree	Feeds on aphids and Plant hoppers	Not so frequent

TABLE 3: Comparison of Fungal species isolated from *Hippophae salicifolia* D.DON

Sl. no	Fungal specimen	Isolated from	Initiation phase: growth of fungal colony (In days)	Final phase: full growth in petri dish (In days)	Colour	Shape of colony	Surface	Dew drop like secretions
1	Cambium fungus	Cambium	04	24	White	Irregular circle	Short, cotton like hyphal mass	Yes
2	Root fungus (R1)	Tap root	02	20	Milk white	Grouped circle	Long, cotton like hyphal mass	No
3	Root fungus (R2)	Secondary root	02	12	Cream	Irregular circle	Short, cotton like hyphal mass	Yes

Table 04: Effect of Insects/ Pests recorded on *Hippophae salicifolia*

Sl.No	Insect / Pests	Effect on Natural Population	Effect on Nursery Seedlings
1.	Altica Beetle	Reduced photosynthesis, stunted growth in seedlings	Stunted growth in seedlings, Causes mortality in Cuttings
2.	Plant Hopper	Reduced fruit yield, Poor growth, May infect plants with diseases	Stunted growth in seedlings, Causes mortality in Cuttings
3.	Aphids	Reduced fruit yield, Poor growth, May infect plants with diseases	Stunted growth in seedlings, Causes dryness and death
4.	Heart wood Borer	Individual was a handsome small tree. May favour fungal diseases	Not recorded in Nursery
5.	Caterpillar	Reduced photosynthesis, stunted growth in seedlings	Stunted growth in seedlings, Causes mortality in Cuttings
6.	Psyllid Bug	Reduced photosynthesis, stunted growth in seedlings	Stunted growth in seedlings
7.	Ground Beetle	Was recorded during the extraction of Frankia nodules	Not recorded in Nursery
8.	Predatory Mites	Reduces pest population by predating on aphids, Psyllids etc	Not recorded in Nursery

ABSTRACT

Name : Charan G S **Id. No.: UUHF/15357**
Year of Admission : August 2015 **Degree : M.Sc Forestry**
Department and major field : M.Sc (Forest Products and Utilization)
Advisor : Dr. V.P. Khanduri (Associate Professor)
VCSG UUHF, College of Forestry, Ranichauri

Thesis title:- "Effect of Entomological and pathological agents on the vegetative propagation, growth and yield of Natural and Nursery population of *Hippophae salicifolia* D.Don"

An investigative study was conducted for one year to investigate the effect of entomological and pathological agents on the vegetative propagation, growth and yield of Natural and Nursery population of *Hippophae salicifolia* D.Don. Invasive pests that gained entry into this cold arid region of India have cost catastrophic damage on commercial agriculture, horticulture crops and forest trees. Out of three fungal pathogens isolated i.e. C1 (isolated from cambium), R1 (Isolated from primary root) and R2 (isolated from secondary root), R2 seems to the fast growing species followed by R1 and C1. Only C1 produced honey dew like droplets on the surface of hyphal mass and showed slight alteration of colour from brilliant white to slightly yellowish white. Though the damage and effects of these fungal species on the vegetative propagation of *Hippophae* cuttings of four month experiment period could not be quantified because of the complexity of the diseases. Only about 4.51% of the 288 cuttings survived as healthy five month old seedlings. The variation of rooting media (8 substrates) or the rooting hormone concentrations (3 ppm concentrations) did not influence any advantage for the *Hippophae* cuttings for healthy and infection free growth. Mountain areas of Yamunothri regions are characterized by inaccessibility, fragility, marginality, diversity, niches and adaptation mechanisms. Insects like Aphids, Psyllid Bug, Heartwood Borer, Altica beetle, Plant Hopper, Phytophagus Caterpillar and Ground Beetle where recorded in association with the *Hippophae salicifolia* in Yamunotri region and Forest Nursery, Ranichauri, either causing considerable damage to the growth or yield or simply associated ecologically as a part of complex web of nature. Predatory mite was also recorded feeding on aphid and plant hopper nymphs. Damage by Aphids and Plant hoppers was observed to be very high due to change in moisture balance of the conduction tissue. With increasing temperature and decreasing rainfall in Yamunotri region, the pest population would only increase.

CHARAN G S (AUTHOR)

VITAE

The author, Mr. Charan G. S, was born on 24th March, 1994, at Gangur, Hassan, Karnataka. He passed his High school and Intermediate examination in 2009 and 2011, respectively from Karnataka Education Board. After that, he completed B.Sc. Forestry from College of Forestry, Sirsi (University of Agricultural Sciences, Dharwad, Karnataka) a ICAR University in year 2015. In 2015, he enrolled in V.C.S.G Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal through Junior Reserch Fellowship (ICAR- AIEEA-PG-2014) for M.Sc. Forestry Degree Programme in Forest Products and Utilization.

Permanent Address:

Charan G S

S/o G B Sanjeeve Gowda

House No- 20/A

Gangur Village at Post

Ramanathapura Hobli

Arakalgud Taluk

Hassan District

Karnataka State - 573133

Mobile No- 8277323215

E-mail ID- charanpeacefulwild@gmail.com