

PEST LIST OF FIVE HIGHLY TRADED MEDICINAL AND AROMATIC PLANTS OF NEPAL



Prepared by
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Nepal Herbs and Herbal Products Association

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Chrysomelid grub on wild asparagus plant (upper left)

Chrysomelid beetle (upper right)

FOREWORD

With the global development of the pharmaceutical industry and progress in chemical techniques, herbal drugs were largely replaced by pure chemical drugs, resulting in a decline of medicinal plant based therapy, particularly in the developed world. However during the recent years, there has been resurgence in the study and use of medicinal plant. Many traditional plant based remedies are back in use, finding increasing application either as a source of direct therapeutic agents or as a raw material base for the development and preparation of chemical compounds.

In particular, the decade has seen a significant increase in the popularity of plant-based medicines. Herbal remedies are increasingly becoming mainstream consumer products manufactured by multinational companies amongst other, and sold in supermarket chains and in a variety of other outlets, globally. A parallel development has been the incorporation of herbs into an increasing number of health foods and dietary products. The combined market is now a multibillion dollar industry in which hundreds of medicinal herbs are being sold in an ever increasing variety of forms. Many plant species used for medicinal purposes also find uses in other fields like aromatherapy, fragrance, essential oils, food supplements, herbal tea, cooking purposes, healing powers, cosmetic purpose, natural coloring, regional cuisines, textile industry, religious ceremonies, etc.

Nepal's biodiversity exists due to its unique climatic conditions, and many isolated topographical locations that host around 7,000 species of plants. Among them, medicinal and aromatic plants (MAPs) have directly contributed to the livelihoods of Nepalese people. Nepal Trade Integration Strategy (NTIS, 2010) has recognized this sector as having export potential and identified some major international markets that allow Nepal to diversify its exports. At present, the processing of MAPs is limited to the distillation of essential oils and preparing few herbal preparations. Essential oils are exported to overseas markets and then used in the manufacturing of cosmetics, perfumes and pharmaceuticals. The major part of collected plants, however, is exported to India and then towards Tibetan Autonomous Region of China in raw form. The regulation of importing countries requires Pest Database of concerned MAPs. This situation has led to impediment in the trade of MAPs sector in Nepal.

Therefore, the effort has been made by the joint collaboration between various government agencies and institutions – basically Ministry of Forest and Soil Conservation, Ministry of

Agriculture Development and Ministry of Industry and Commerce in undertaking "*Preparation of Survey Surveillance Guideline and Pest Database for Selected Most Five Traded Medicinal and Aromatic Plants (MAPs) of Nepal*". In order to facilitate and coordinate among government agencies and concerned stakeholders, NEHHPA has taken a lead to initiate and carry out the activities with the financial support of GIZ/Include-TPP. Preparation of pest database of MAPs is also one of the major activities identified by Project Advisory Group (PAG) consultation workshop during the preparation of Action Plan for the implementation of MAPs project by GIZ-WTO/EIF-SP during 2011-2012. The aim was to generate a comprehensive list of sources of risks and events that might have an impact on the achievement of each of the objectives identified in the context. These events might prevent, degrade, delay or enhance the achievement of those objectives.

In the present survey and surveillance study, economically important five MAPs namely *Asparagus racemosus* Willd. from Makwanpur district, *Swertia chirayita* (Roxb.) H.Karst. from Ilam, *Zanthoxylum armatum* DC from Salyan, *Sapindus mukorossi* Gaertn. from Darchula and *Neopicrorhiza scrophulariiflora* (Pennell) D.Y.Hong from Jumla were collected for the study of fungal and bacterial disease

The coordination of NEHHPA and NEHHPA president Mr. Govinda Ghimire is highly appreciated. We highly commend the contributions of entomology expert Mr. Sanjay Bista and pathology expert Ms. Sangita Joshi in preparing the pest list. The concept and coordination Ms. Rose Shrestha, Scientific Officer, Ms. Jyoti Joshi Bhatt, Chief NPRL, DPR and Ms. Himadri Palikhe, GIZ/TPP is highly acknowledged. The hard effort put into this research by staffs of Biological Section, DPR, particularly Ms. Shiwani Khadgi, Mr. Pramesh Bahadur Lakhey, Ms. Suprava Shrestha, Ms. Yamini Panta and Mr. Dambar Bahadur Karki from MAPs promotion section, DPR was instrumental in completing this project. Likewise the contribution of Mr. Prakash Paudel and Mr. Madhav Lamsal, Plant Pathologists (NPQP) are highly acknowledged.

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We hope that this publication will provide information for risk assessment and management as well as trade promotion of MAPs.

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This publication is an initiation of Nepal Government to comply with WTO rules and commitments under the principles of transparency in WTO agreement, along with many other obligations to facilitate trade. Partner countries are obliged to inform trade partners and/or neighbors of their pest status. Therefore, it is necessary to prepare and compile pest data base of the important MAPs commodities of Nepal.

This publication is the joint outcomes of the collaboration of various individuals and organizations. The overall support and collaboration from Department of Plant Resources (DPR), National Plant Quarantine Program (NPQP), Nepal Herbs and Herbal Products Association (NEHHPA) is highly acknowledged. We would like to thank these organizations for providing human as well as physical resources. The financial as well as technical support from GIZ-INCLUDE/TPP Nepal is appreciated.

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The Study Team

ACRONYMS

CABI:	Commonwealth Agriculture Bureau International
CA:	Catalase Test
CDO:	Chief District Officer
CFT:	Carbohydrate Fermentation Test
CIT:	Citrate Utilization Test
DFO:	District Forest Office
DNA:	Deoxyribonucleic Acid
DOA:	Department of Agriculture
DPR:	Department of Plant Resources
DPRO:	District Plant Resources Office
FAO:	Food and Agriculture Organization
FNCCI:	Federation of Nepalese Chamber of Commerce and Industry
GIZ:	German Technical Cooperation
GPS:	Geographical Positioning System
IND:	Indole Test
IPPC:	International Plant Protection Convention
ISPM:	International Standard for Phytosanitary Measures
IUCN:	International Union for Conservation of Nature
MAPs:	Medicinal and Aromatic Plants
MOAD:	Ministry of Agriculture Development
MOFSC:	Ministry of Forests and Soil Conservation
MR:	Methyl Red Test
NA:	Nutrient agar
NARC:	Nepal Agricultural Research Council
NAST:	Nepal Academy of Science and Technology
NEHHPA:	Nepal Herbs and Herbal Products Association
NHM:	Natural History Museum
NI:	Nitrate Reduction Test
NPPO:	National Plant Protection Organization
NPQP:	National Plant Quarantine Program
NTIS:	Nepal Trade Integration Strategy
OXI:	Oxidase Test
PAG:	Project Advisory Group
PDA:	Potato Dextrose Agar
PPD:	Plant Protection Directorate
PRA:	Pest Risk Assessment
PSA:	Potato Sucrose Agar
SPS:	Sanitary and Phytosanitary Measures
TPP:	Nepal-German Trade Promotion Programme
TWA:	Tap Water Agar
URE:	Urease Test
VDC:	Village Development Committee
VP:	Voges-Proskauer Test
WTO:	World Trade Organization

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EXECUTIVE SUMMARY

It has been estimated that about 1,463 species of medicinal and aromatic plants (MAPs) are used by rural people of Nepal. Collection and trade of MAPs have directly contributed to the livelihoods of people in Nepal's hilly and mountainous areas. The Nepal Trade Integration Strategy (NTIS, 2010) has recognized the MAPs sector as one of the commodities for export promotion. The major part of collected MAPs is exported to India and then to China in crude form.

For last few decades; with the threat of different pests; the import and export of MAPs and other plants based products in crude forms are highly regulated. Till date, Plant Quarantine Office has been issuing Phytosanitary Certificate for the export of MAPs on the basis of traditional methods of observation. Declaration of pest freedom in Phytosanitary Certificate requires national pest database accompanying with detection protocol of quarantine pests concerned to importing contracting party. On the other hand, the obligations of importing countries require exporting country's pest database for undertaking PRA to determine their quarantine pests. While undertaking PRA by the importing countries, the exporting country must supply the information as needed to them.

Thus sensing the economic importance and the need to prepare pest database for export of concerned MAPs, Nepal Herbs and Herbal Products Association (NEHHPA) along with Department of Plant Resources (DPR) and National Plant Quarantine Program (NPQP) with the financial support of Nepal-German Trade Promotion Programme (TPP) have prepared the pest database of highly traded MAPs during the project period till 2015 AD.

There are more than 100 MAPs that are in trade in raw form and it is impossible to carry out the survey surveillance to prepare pest database for all those commodities. Thus only five MAPs were selected, primarily on the basis of traded volume (mostly in raw form) and as per the expert consultation. Also the associated districts were selected as per the distribution and trade volume.

The current study was focused on five highly traded MAPs from different ecological zones namely *Asparagus racemosus* Willd.) from Makwanpur district (Tarai/Siwalik), *Swertia chirayita* (Roxb.) H. Karst. from Ilam (mid hills), *Zanthoxylum armatum* DC (mid hills) from Salyan, *Sapindus mukorossi* Gaertn. from Darchula (mid hills) and *Neopicrorhiza scrophulariiflora* (Pennell) D.Y.Hong from Jumla (high hills) were collected for the study of fungal and bacterial disease

The project was initiated by preparing the roster of experts, followed by formulating the expert team (comprising the team leader/ ecologist, pathologist, entomologist and research associate). This was followed by an eight days training for 15 participants who were identified to undertake the field study. The host specific pest detection survey plan was prepared for all five selected MAPs. The guideline was duly approved by NPPO and the final version was published by NPQP in November 2014. Afterwards, generic survey surveillance guideline entitled "Technical guidelines for detection survey of plant pests in Nepal, 2014" was prepared by NPPO.

Field visits were planned according to the host specific pest detection plan and generic survey surveillance guideline. It was agreed to carry out three consecutive visits (seedling/ growing, flowering/ fruiting and harvesting/ post harvesting stage) to collect and study all possible pest of the commodities.

Samples were collected following the stratified and simple random sampling method. Full sample observation was undertaken on all randomized sub plots until the species accumulation curve was satisfied. Clustered locations were randomly sampled according to horizontal distribution and altitudinal gradients. Field survey work was accomplished as per the as per the protocol mentioned in the NPPO survey guidelines. Storage conditions and associated pests in the storage facilities of the farmers, local road-head collectors and traders were observed and studied to obtain information on the storage practices, conditions and pests.

The collected samples were transported to Biological Section at DPR and pest identification were carried out following mycological (isolation of organisms, single spore culture of isolated species and identification of organisms), bacteriological (isolation and purification of bacterial isolates and identification of bacteria), entomological and weed identification (reference sample) method.

During the survey, the studied MAP commodities were found to be affected by number of pests during different crop stages in varying intensities. The pests were broadly divided into three different groups as diseases, insects and weeds. Among the commodities, the highest numbers of pests were observed in Kurilo (16), followed by Rittha (14), Chiraito (13) and Kurilo and Timur (12). The prevalence of diseases in the commodities was comparatively more than other pests (insect and weed). Incidence of some pests seems to be generalized while some are highly species specific. The infestation of weed was not found as severe as that of disease and insects. Some

pests are identified up to generic level which some unidentified pests are in process of identification.

CHAPTER I: BACKGROUND

1.1 Introduction

Nepal's biodiversity is a reflection of its unique geographical position, undulating landscape, as well as altitudinal and climatic variations. As a result, Nepal hosts around 6500 species of plants (Hara *et. al*, 1979), out of which 1463 species are used by rural people of Nepal (MoFSC, 2006) collectively known as medicinal and aromatic plants (MAPs). MAPs have directly contributed to the livelihoods of people especially in mountainous and hilly areas for many centuries. The MAPs sector has high potential to contribute in growing international trade. The Nepal Trade Integration Strategy (NTIS, 2010) has recognized this sector as one of the commodities for export promotion. The major part of collected plants is exported to India and then to China in raw form and very few percentage is processed into essential oils that are exported to overseas markets to be used in cosmetics, perfumes, and pharmaceuticals. During the transport, they are prone to risk of transferring pests. Since last decade, plant health status has become a major trade-policy issue because of the threat of disease and pests and therefore, the import and export of MAPs and other plants based products in crude forms are highly regulated.

Nepal's accession to the World Trade Organisation (WTO) in 2004 offers significant opportunities for the country to foster trade for development and improve the investment climate and good trade governance. During the process of accession, Nepal has however also entered into significant obligations when it committed to comply with WTO rules and commitments, to adjust its laws and to create and strengthen the necessary infrastructures. In this framework, the country has to fulfill many obligations while exporting and importing the agricultural goods and allied plant materials. The issuance of Sanitary and Phytosanitary certificate (SPS) while importing and exporting the goods is primary requisite. At the same time importing country should conduct pest risk analysis (PRA) for bio-security to check probable risk of pest infestation. Likewise exporting countries are obliged to provide necessary information about pest list of concerning commodities.

Requirement under International Agreement

A pest list is a requirement under the International Plant Protection Convention (IPPC), Article IV/2 (b), which states that the responsibilities of an official National Plant Protection Organization shall include the following: the surveillance of growing plants, including both areas under cultivation and wild flora, particularly with the object of reporting the occurrence of pests, and

Article VIII/2 (b) which states that the contracting parties shall cooperate with one another to the fullest practicable extent in achieving the aims of this Convention, and shall in particular: cooperate in the exchange of information on plant pests, particularly the reporting of the occurrence, outbreak or spread of pests that maybe of immediate or potential danger, in accordance with such procedures as may be established by the Commission.

Pest List Database (PLD) is also required under obligations of the WTO Agreement on the Application of SPS by Article 5: Assessment of Risk and Determination of the Appropriate Level of Sanitary or Phytosanitary Protection, Article 6: Adaptation to Regional Conditions, Including Pest- or Disease-Free Areas and Areas of Low Pest Prevalence. Article 6/3 states: "exporting members claiming that areas within their territories are pest_free areas or areas of low pest prevalence shall provide the necessary evidence thereof in order to objectively demonstrate to the importing member that such areas are, and are likely to remain, pest free areas or areas of low pest prevalence, respectively. For this purpose, reasonable access shall be given, upon request, to the importing member for inspection, testing and other relevant procedures."

Pest Risk Analysis is the process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it. For this, countries negotiating trade in agricultural commodities that may provide pathways for moving pests into new areas must be able to access information on the biology, distribution, host range and economic status of plant pests under principles of transparency enshrined in IPPC agreement on application of Sanitary and Phytosanitary (SPS) measures. They are obliged to inform trade partners and/or neighbors of their pest status. It is therefore necessary to prepare and compile database of field and storage pests including insect, mites, pathogens, weeds, etc. of the important commodities that are potential for international trade. National Plant Protection Organization (NPPO) will be responsible to disclose and keep records of such information. General survey and surveillance is the tool used to gather such information.

Therefore, the effort has been made by the joint collaboration between various government agencies and institutions, basically DPR/MoFSC, NARC and NPQP in undertaking "*Preparation of Survey Surveillance Guideline and Pest Database for Selected Most Five Traded Medicinal and Aromatic Plants (MAPs) of Nepal*". Nepal Herbs and Herbal Products Association (NEHHPA)

lead the consortium to facilitate and coordinate among government agencies and concerned stakeholders while GIZ/Include – Trade Promotion Project provided the financial support. Preparation of Pest Database of MAPs is also one of the major activities identified by Project Advisory Group (PAG) consultation workshop during the preparation of Action Plan for the implementation of MAPs project by GIZ-WTO/EIF-SP during 2011-2012. Government of Nepal has also prioritized this activity in their Procedure on Forestry Sector Operational Plan (2013).

1.3 Objectives

The major objective of this assignment is to facilitate the export of crude Nepalese MAPs by preparing the pest database.

The specific objectives are as follows:

- Preparation, publication and endorsement of “Technical Guidelines for Detection Survey of Plant Pests in Nepal”,
- Preparation and publication of pest list on five highly MAPs namely Gentian (*Neopicrorhiza scrophulariflora*), Chireeta (*Swertia chiraytia*), Prickly Ash (*Zanthoxylum armatum*), Soapnut (*Sapindus mukorossi*) and Asparagus (*Asparagus racemosus*)

1.4 Rationale

There are over more than 1463 MAPs in Nepal that are commonly used for medicinal purposes, both for traditional and modern health care. The value of MAPs has been recognized widely with its increasing contribution to the Nepalese economy (Edwards, 1996) where more than 50% of the population of some hilly and mountainous area are engaged in trade and it may contribute 10-100% income of rural communities (Olsen and Larsen, 2003).

MAPs and herbs based products are one of the major export items from Nepal. Nepal's export of MAPs and products estimated over NRs. 2.5 billion per year contributing 4% of the total contribution of forestry sector to the national economy (referred from Gurung, 2013). In FY 2013/014, crude medicinal plants worth NRs. 1,602.19 million was exported out of NRs. 2,414.96 million export from medicinal plants, essential oils and handmade papers, a 25.9% increment in monetary value compared to FY 2012/013 (www.tepc.gov.np). More than 80% crude MAPs are exported to India and China in terms of value and volume (referred from Gurung, 2013) indicating that crude MAPs is one of the important exporting commodity of Nepal.

Trade of crude MAPs from Nepal to India and China has commenced since time immemorial and the trade process was much easier those times. But for last few decades, with the threat of disease, insects and other pests, the import and export of MAPs and other plants based products in crude forms are highly regulated. Till date, Plant Quarantine Offices has been issuing Phytosanitary Certificate for the export of MAPs on the basis of traditional methods of observation. Declaration of pest freedom in Phytosanitary Certificate requires national pest database accompanying with detection protocol of quarantine pests concerned to importing contracting party. On the other hand, the obligations of importing countries require exporting country's pest database for undertaking PRA to determine their quarantine pests. While undertaking PRA by the importing countries, the exporting country must supply the information as needed to them. The information is asked through PRA request form and the form is sent to the NPPO. The information asked by the importing country must be generated by exporting country with the help of ongoing surveillance program. Nepal, till the commencement of this project, did not have her national pest database and any pest detection protocol of the related MAPs. This situation has casted an alarm to pose some impediments in the trade of MAPs sector in Nepal. Therefore, it was sought urgent to generate national pest database through the field based survey. As mentioned already, there were no any survey guidelines and pest database prepared earlier, preparation of detection survey guideline and pest database of traded MAPs to facilitate the trade and to ensure the optimum benefit to the actors involved in the supply chain of MAPs is imperative.

Literally, Ministry of Agriculture Development (MoAD) is responsible to prepare the pest database but with the limited human and financial resources, MoAD is focusing only on agricultural crops. Preparing pest database for MAPs is not even in their action plan. Thus sensing the economic importance and the need to prepare pest database for export of concerned MAPs, Nepal Herbs and Herbal Products Association (NEHHPA) along with Department of Plant Resources (DPR) and National Plant Quarantine Program (NPQP) with the financial support of Nepal-German Trade Promotion Programme (TPP) wished to prepare pest database of five highly traded MAPs during the project period till 2015 AD.

1.5 Limitations

There are approximately 100 MAPs traded in and from Nepal but about one third i.e. 32 MAPs products (list in Annex I) have significant contribution in terms of value and volume of trade

(Olsen, 2005). More than two dozen countries are reported to import MAPs from Nepal, the major being India, China, Pakistan, Bangladesh, Singapore, Japan, Australia, USA, Canada, Germany, Belgium, Italy and so on (Gurung, 2013), reflecting that all the MAPs are important and Nepal have to prepare pest database for at least three dozen MAPs (28-30 MAPs from the old list and few others whose trade have escalated in recent years). Preparation of pest database of these MAP species is possible only through field based surveys. Almost all the field based studies in the area of plant pest surveillance faces few or more limitations. These limitations sometimes become unavoidable with the prevailing circumstances few of which were: smaller coverage with respect to area, relatively short duration of overall study, inadequate access to quantitative data such as disease severity or intensity of insect infestation, unavailability of few but highly required qualitative data such as specimen intact attacking stem of Chireeta.

Preparation of pest database for all aforementioned MAPs is lengthy and time consuming process and it also needs substantial financial and human resources. The financial requirement can be managed but it was very difficult to manage the human resources. Because of the leave requirements of donors, leave unavailability of the consultants and the frequent travel need of the field surveying system (at least for three seasons for one species), followed by time consuming laboratory analysis procedures, the duration of field survey schedules were curtailed all the time. Aggravating this, the Trade Promotion Project ends on 2015, leaving only two years for project completion. The assignment has prepared pest database for five highly traded MAPs, although the database generated and presented in this report is just a year index of the pest status of limited areas. Further, no any measurable data about the severity and intensity of the pest attack in surveyed commodities were calculated, because the survey was focused only for detection of pests. Although, PRA requires several information about loss assessment and pest biology, no data about severity and intensity of pest attack was calculated during this survey. Also, the biology of the pest attacking surveyed commodities was not studied.

Initially fifteen survey surveillance visits was proposed (three consecutive visits for five species during sprouting, growing/flowering and collection season) but it was decreased to thirteen because of the difficulty in managing the time of expert human resources (case of Chireeta at Ilam), and harsh climatic condition at Jumla during November, 2014. Thus survey of Gentian and Chireeta was carried out only during sprouting and growing season.

The larvae of almost all pests need to be reared to mature stages in laboratory for proper identification. But the team had difficulty in transporting these larvae in regulated temperature due to inadequate technology. Further, the pests collected were studied in the laboratories of DPR, the pests identification is not verified yet and there were not sufficient live sample to identify and thus the final list of pest database cannot be considered complete. During the identification process, some specimens could be identified up to genus level. During the field visit, some specimens could not be collected at the field but the damage symptoms were prominently observed; these pests are described as per the symptoms observed at the field.

Pest data list should include weeds also. But due to inadequate time and resources, weed collection could not be done in this assignment. Only few cases of associated plant species came across with targeted species are listed out. Detail study about problematic weed species and parasitic plants associated with targeted species is needed. Other pests like birds, rodents, molluscans, etc were also excluded.

CHAPTER II: STUDY AREA AND METHODOLOGY

2.1 Review of the literatures, acts and regulations

The relevant literature when and wherever available was collected and placed at DPR office. The reports available at concerned offices were collected and other related materials were also retrieved from internet. The Crop Protection Compendium from CABI International and other official documents were referred as and when required. The acts and regulations (Plant Protection Act and Regulation, 2064) enforced by Government of Nepal regarding the current work was also referred.

2.2 MAPs and districts selected for survey

2.2.1 Preliminary list of MAPs with proposed survey districts

The expert team carried out literature review, series of consultation meeting/workshops and consulted concerned experts to select the five most traded MAPs. During the process of selection, the team first prioritizes twenty-two MAPs that are mostly traded in raw form, on the basis of trade value and volume, revenue generated and geographical distribution (Table 1).

Table 1: List of proposed MAPs for pest database preparation

SN	Botanical name	Common name	Parts in trade	Proposed areas of collection and survey
1	<i>Aconitum heterophyllum/ Delphinium himalayai</i>		Rhizome/ root	Jumla/ Mugu
2	<i>Asparagus racemosus</i>	Wild Asparagus	Rhizome/ root	Makwanpur
3	<i>Berberis asiatica/ Berberis aristata</i>		Stem and root bark	Nuwakot/ Rasuwa
4	<i>Bergenia ciliata</i>	Rockfoil	Rhizome	Nuwakot/ Rasuwa
5	<i>Cinnamomum tamala</i>	Cinnamom	Leaf	Jhapa/ Nawalparasi/ Udayapur
6	<i>Elaeocarpus sphaericus</i>		Seed	Sankhuwasabha/ Bhojpur
7	<i>Fritillaria cirrhosa</i>	Fritillary	Bulb	Jumla/ Mugu
8	<i>Juniperus indica/ Juniperus communis</i>	Juniper	Needle	Jumla
9	<i>Morchella conica/ Morchella esculenta</i>	Morchella	Whole plant	Jumla/ Mugu
10	<i>Nardostachys grandiflora</i>	Spikenard	Rhizome/ root	Jumla/ Mugu
11	<i>Neopicrorhiza scrophulariiflora</i>	Gentian	Rhizome/ root	Jumla/ Mugu
12	<i>Paris polyphylla</i>	Love Apple	Rhizome/ root	Ilam
13	<i>Persea odoratissima</i>		Bark	Doti/ Dadeldhura
14	<i>Rauvolfia serpentina</i>	Serpentine	Rhizome/ root	Makwanpur

15	<i>Rheum australe</i>		Rhizome and stem	Rasuwa
16	<i>Rubia manjith</i>		Stem and root	Nuwakot
17	<i>Sapindus mukorossi</i>	Soapnut	Fruit	Doti/ Dadeldhura
18	<i>Swertia chirayita</i>	Chireeta	Whole plant	Ilam/ Panchthar
19	<i>Taxus wallichiana</i>	Himalayan Yew	Leaf	Jumla
20	<i>Valeriana jatamansii</i>	Valerian	Rhizome/ root	Jumla/ Mugu
21	<i>Zanthoxylum armatum</i>	Prickly Ash	Fruit	Salyan/ Rolpa
22	<i>Parmelia nepalensis</i>	Lichen	Whole plant	Makwanpur

2.2.2 Final list of MAPs with survey districts

From these twenty-two MAPs, the team, with further expert consultation as well as reference review selected five MAPs for the survey purposes on first year. The districts to be surveyed for these five targeted MAPs were selected on the basis of their distribution and availability to collect field information and pest samples (Table 2).

Table 2: Selected MAPs and districts for survey, 2014

SN	Selected MAPs		Selected districts	Code no.
	Scientific name	Common/ Local name		
1	<i>Asparagus racemosus</i> Willd.	Wild Asparagus	Makwanpur	Mak
2	<i>Swertia chirayita</i> (Roxb.) H. Karst.	Chireeta	Ilam	Ila
3	<i>Zanthoxylum armatum</i> DC	Prickly Ash	Salyan	Sal
4	<i>Sapindus mukorossi</i> Gaetrn.	Soapnut	Darchula	Dar
5	<i>Neopicrorhiza scrophulariiflora</i> (Pennell) D.Y.Hong	Gentian	Julma	Jum

2.2.3 Species background

Soapnut, *Sapindus mukorossi* Gaetrn.

Sapindus mukorossi Gaetrn., locally known as Ritha, Aritha, Dodan, Doadni, Doda, Kanma and Thali in different Nepali dialects. The plant belongs to family Sapindaceae of order Sapindales.

It is distributed throughout Himalayan range of NE India, Indo-China, Myanmar, Taiwan, Korea and Japan. In Nepal, although it has been reported to occur from 600-1400 masl throughout hills and plains, it commonly grows in the western parts Nepal.

It is a medium sized tree. Full and mature plant is up to 15 m tall. Leaves are alternate, sometimes odd-pinnate leaflets 8-15 in number, lanceolate, glabrous, alternate or the upper ones nearly opposite. Flowers are numerous, small, polygamous, purple, in terminal, pubescent, pyramidal panicles and are 10-20 cm long.

The fruit are rounded, fleshy drupes, measuring 1.5-2.5 cm in diameter; it is light brown after drying, somewhat translucent saponaceous rind with a wrinkled surface; the drupe encloses single globose black seed with smooth surface and hard pericarp.

The leaves turn yellow in December before being shed in December-January. Tree delicious with leaf appearing on March-April. The panicles of white or purplish bisexual flowers appear in May-June, with the green fruits ripening in October-November. These remain on the tree till January or later.

Recently domesticated and cultivated by farmers, Soapnut has become a highly traded medicinal plant of Nepal. Its fruit, known as soapnut, is sold either as entire fruit or fruit shells or as shell powder. It is used as the main ingredient in soaps and shampoos for washing hair, as it is considered good for the health of hair. The trend of washing hair with Soapnut is still followed in many local households. The jewelers in India use this plant to bring back the lost brightness of ornaments made of precious metals like gold, silver, etc. The herb is also used in the treatment of extra salivation, migraine, epilepsy and chlorosis.

It is commonly cultivated in western Nepal in Darchula, Dadheldhura and Bhajhang districts. It is locally sold at the rate of around NRs. 15-25 per kg. As prescribed by the Forest Regulation, 1995 and its third amendment, 2005, Government royalty of fruit and seed of *S. mukorossi* is NRs. 2/25 kg of fruit and NRs 0.15/kg of nut.

Wild Asparagus, *Asparagus racemosus* Willd.

Asparagus racemosus Willd. commonly known as Wild Asparagus and locally known as Kurilo or Satawari belongs to family Asparagaceae of order Liliales.

It grows in Bhutan, China (Tibet), India (Kashmir, Sikkim), Myanmar, Nepal (Central and Western) and Pakistan. It is generally found in open slopes, forests and shrubberies within the altitudinal range of 300-2200 masl occurring mostly from 500 to 1300 masl. Naturally, it can be found mostly in community forests, leasehold forests, national parks and conservation areas.

A. racemosus is a xerophytic perennial shrub. Its stem is woody with stiff spines at the base bending towards ground. The leaves are needle-like cladodes. It bears tiny white flowers in raceme which develop into green berries turning red upon ripening.

A. racemosus has been successfully cultivated in various districts of Nepal like Makwanpur, Chitawan, Bara, Parsa, Sindhupalchowk, Kavrepalanchowk, Myagdi, Gulmi, Nawalparasi, etc.

It is a highly traded medicinal plant. It has been domesticated and cultivated recently by farmers for tuberous root, the traded part of the plant. Government royalty of the tuber of *A. racemosus* is NRs. 5/ kg.

Chireeta, *Swertia chirayita* (Roxb. ex Fleming) H. Karst.

Swertia chirayita (Roxb. ex Fleming) H. Karst. locally known as Chiraito or Tite, belongs to family Gentianaceae of order Gentianales.

The species is endemic to the Himalayas and is distributed from Kasmir through Nepal and Sikkim NE India to Bhutan. In Nepal, it occurs from east to west throughout the foothills of the Himalayas within the altitudinal range of 1500 to 3000 masl. It grows in open and moist places, forest floor and edge of agricultural lands. It prefers north- and north-west facing moist habitats in forests, rangelands and around cultivated lands, but is mostly found on south-west facing slopes of mixed broad-leaved forests. It is mostly found in black sandy soil rich in organic matters and acidic with pH 4.5-5.5 and adequate soil moisture.

The plant is a perennial erect herb, about 60-125 cm tall with robust branching in later stage. The plant develops as rosette form and in the second year develops elongated flowering and fruiting branches in course of its growth-cycle. Root is somewhat twisted, 5-10cm long, 1-2 cm in diameter, gradually tapering downwards, solid, light brown to purple brown in color. Stem is quadrangular towards upper portion and whole plant is bitter in taste. The flowers are tiny purplish to brown in color: insects being attracted in purple color. Fruits are capsules ca. 0.6 cm, ovoid and seeds are small, numerous.

Chiretta has been a popular broad spectrum herb. All parts of the plant including leaves, flowers, stem and roots are used in medicine. It is used for numerous purposes including as a bitter tonic, to increase appetite, control malarial and other types of fever, hepatitis, inflammation, burning sensation during urination, etc. Highly traded medicinal plant, *S. chirayita* constitutes about 80 percent of the total traded volume in the country. About 90 percent of the Chiretta produced in the country are exported, about 80 percent to India and variable quantities to other countries including China, Malaysia, Singapore, Germany, Italy, France, Switzerland, Sri Lanka, Bangladesh,

Pakistan, USA and many others. Recently it has been domesticated and cultivated by farmers. Whole plants are used to extract its medicinal constituents, bitter principles for commercial purposes. *S. chirayita* has been categorized as 'vulnerable' species based on the IUCN Threat Categories-version 3.1 (Bhattarai *et al.*, 2002). Government royalty of *S. chirayita* (harvested from wild) is NRs. 15 kg.

Prickly Ash. *Zanthoxylum armatum* DC

Zanthoxylum armatum DC is locally known as Timur. It is found between 1100 to 2500 masl throughout Himalayan range from (Kashmir to Bhutan), N. India, China, Taiwan and Philippines (Press *et al.*, 2000). It grows mostly in moist areas with deep soils and sites, exposed to sun and in degraded slopes, shrub lands, natural forests and wastelands. The plant grows as a shrub or sometimes a small tree about 6 m tall, with corky bark and with numerous long straight spines on branchlets. The flowers are about 1 mm, one-sexed; calyx with 6-8 acute lobes; petals absent; stamens 6-8, much longer than calyx in male flowers. Fruits are follicles initially green, becoming purplish red when ripe and bearing a few protruding oil glands. Each fruit contains a blackish-brown seed (Bhattarai and Ghimire, 2006; DPR, 2007; Polunin and Stainton, 1984).

The dried fruit is a popular Nepalese spices used in pickle and vegetable especially mushroom preparation. Due to appealing aroma and multipurpose medicinal properties present in its fruit and bark, Prickly Ash is used in the manufacture of several health-care and cosmetics as well as toiletries products. India is the major commercial outlet for dried timur fruits (Edwards, 1996; Den Hertog, 1997). Estimated demand in 2004/05 in Indian sub-continent of timur was 23 MT but only 10 MT could be supplied annually. Domestic market is also remarkable with 5500 kg in Kathmandu. Government royalty of *Z. armatum* is NRs. 8/ kg.

Gentian, *Neopicrorhiza scrophulariflora* (Pennell.) D.Y. Hong

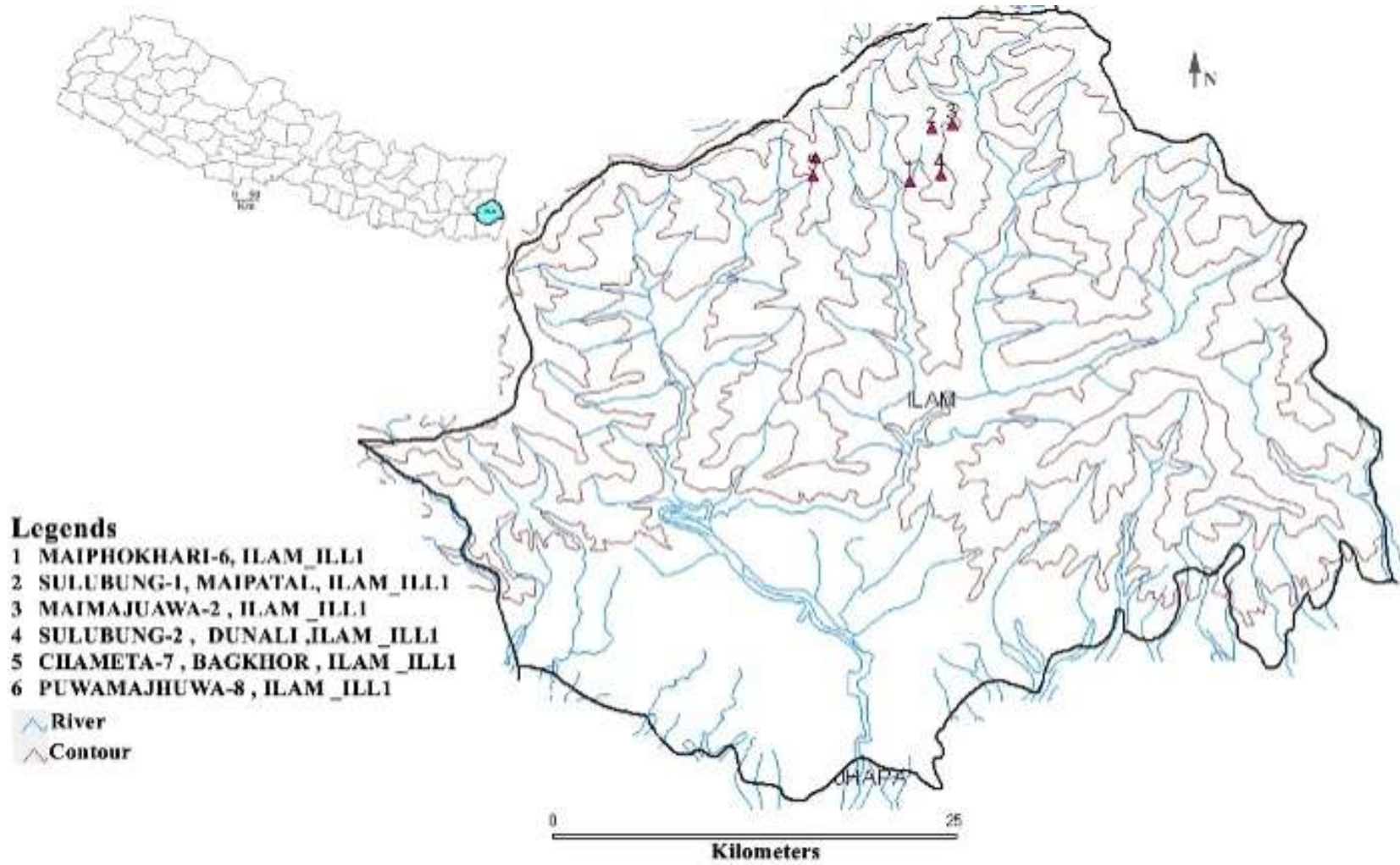
Neopicrorhiza scrophulariflora (Pennell.) D.Y. Hong, locally known as Kutki or Katuki in Nepali is found in alpine grassland, gravelly areas in the Himalayas, from Nepal to Bhutan, China, NE India, Myanmar, at altitudes of 3500-4800 masl.

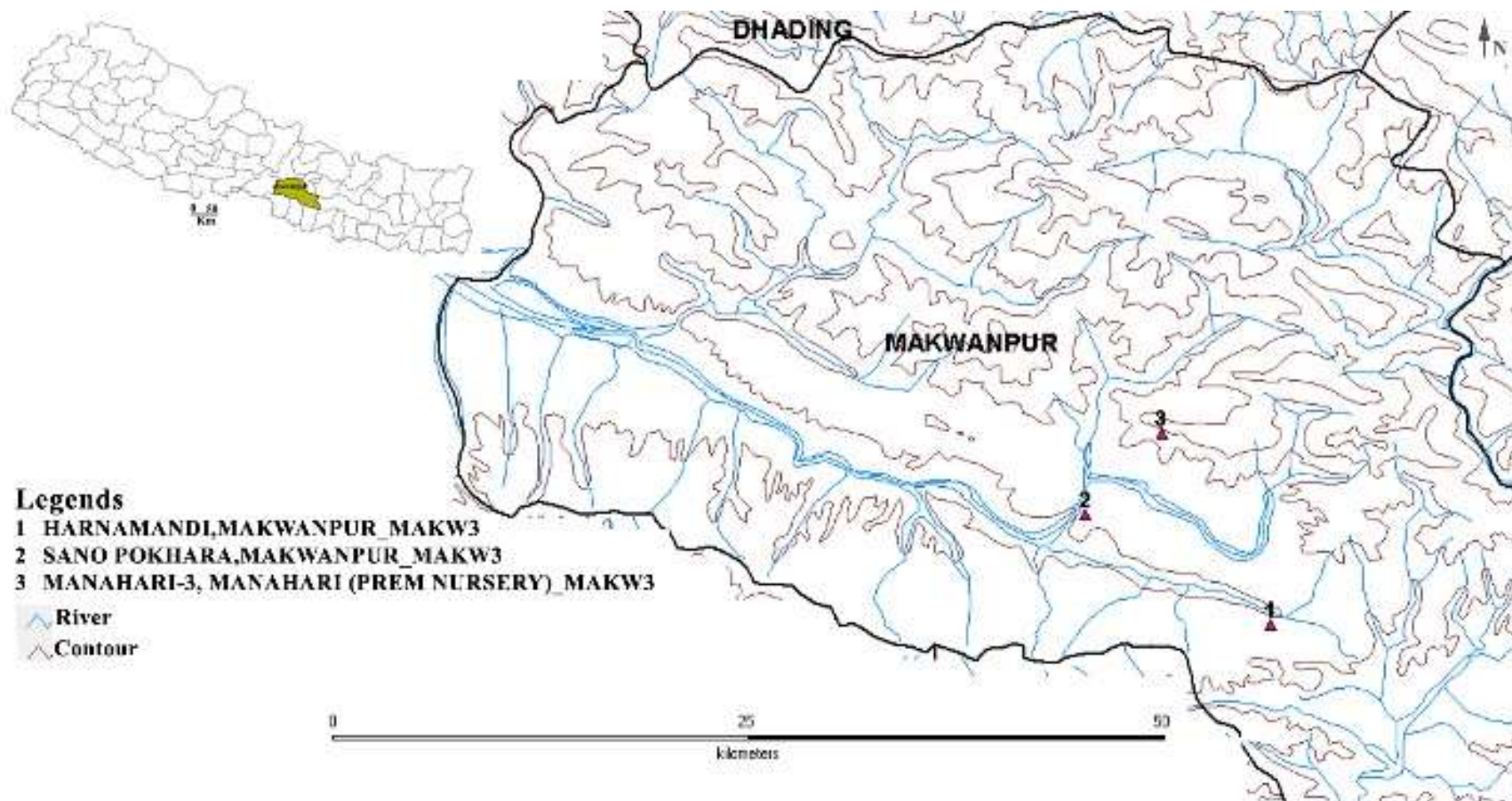
The plant is a perennial herb, 4-12 cm tall with rhizomes up to 1 cm in diameter, coarsely rooting from nodes. Leaf-stalks are short, leaves spoon-shaped to ovate, 3-6 cm long, black when dry, base tapering, margin toothed or rarely double toothed. Flowering stem is brown glandular hairy.

Flower spike 1-2 cm, flower-stalks are 2-3 mm, sepal cup is 4-6 mm, up to 1 cm in fruit, sepals lanceshaped to obovate-oblong, upper sepal linear, brown glandular hairy. Flowers are dark purple, 0.8-1 cm, outside velvety, tube 2-3 mm to 4-5 mm, lower lip about 1/2 as long as upper, 3-lobed, lateral lobes with 2 or 3 small teeth. Upper lip is hooked, emarginate. Capsules are narrowly ovoid, 8-10 mm.

The flowering occurs in May - August while fruiting occurs from August – October. Rhizomes and roots are used to treat high blood pressure, fever, bile and other intestinal pains and cold and sore throats. Government royalty of *N. scrophulariiflora* is NRs. 15 kg.

2.2.4 Map of survey districts with GPS data code

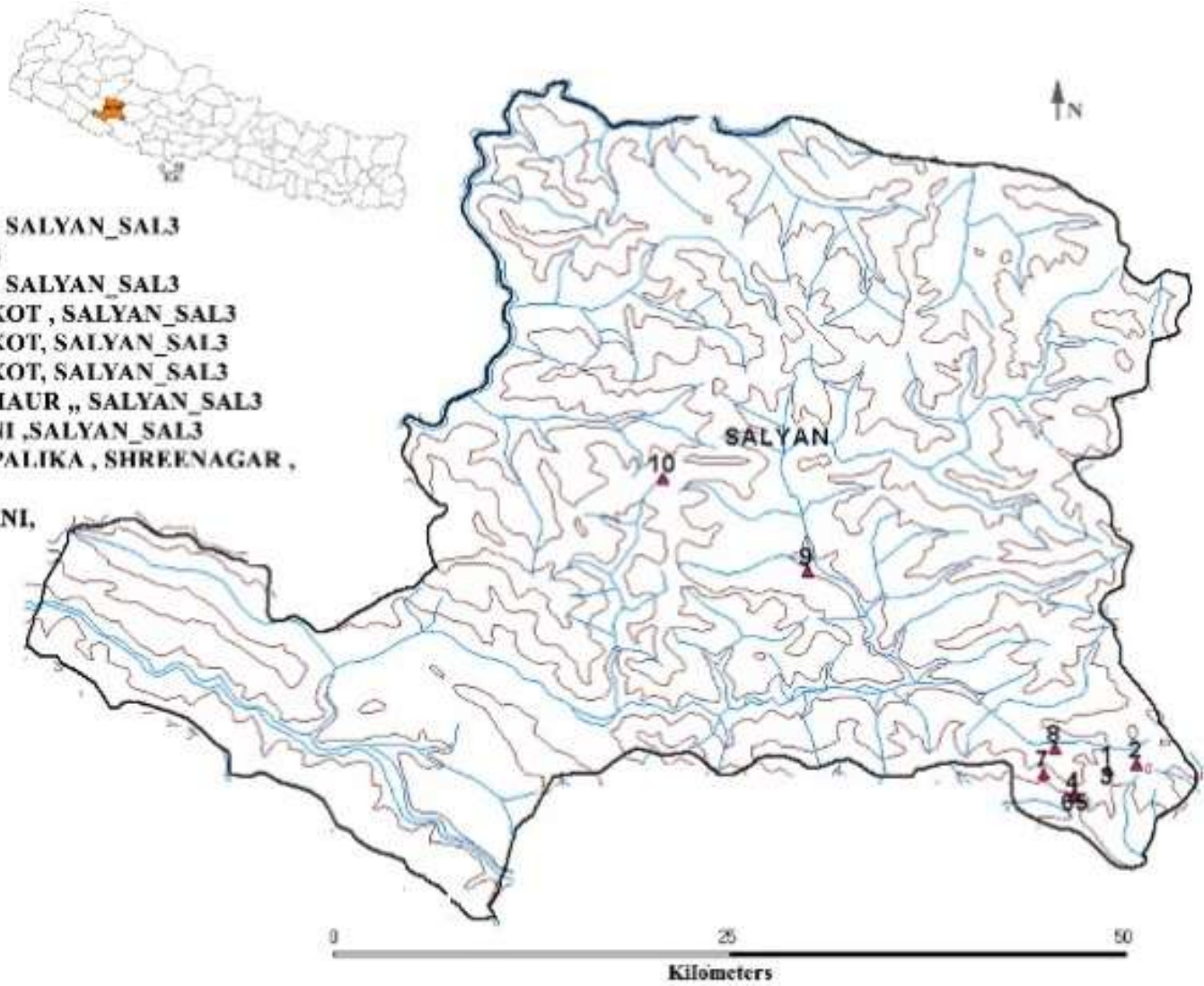


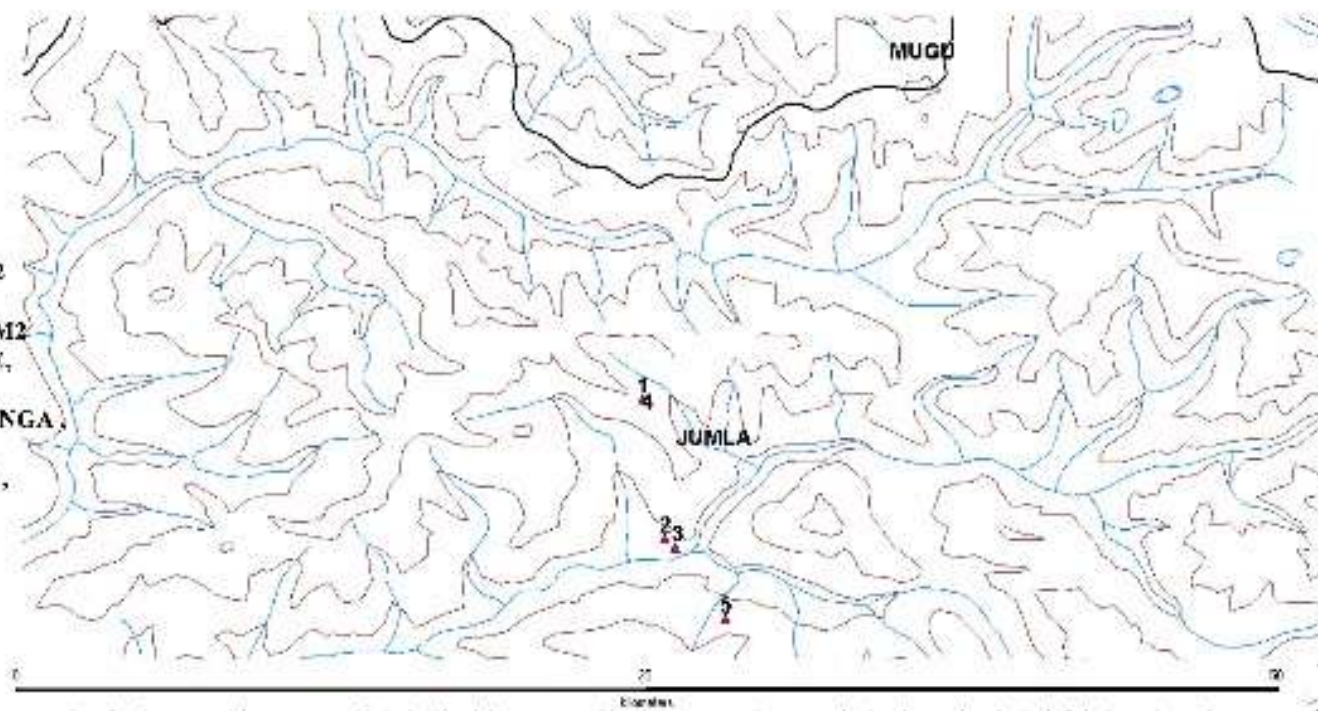
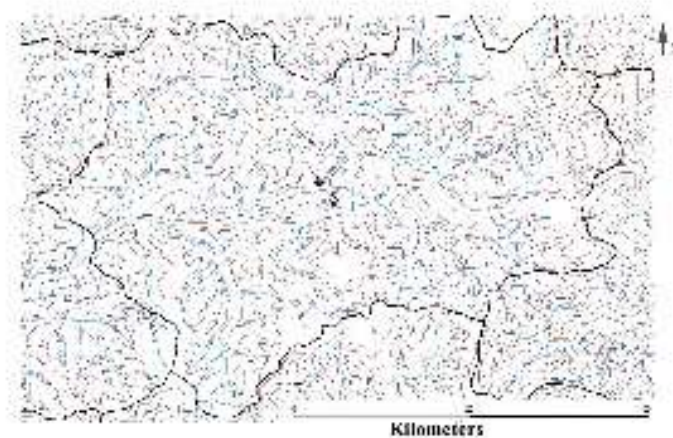
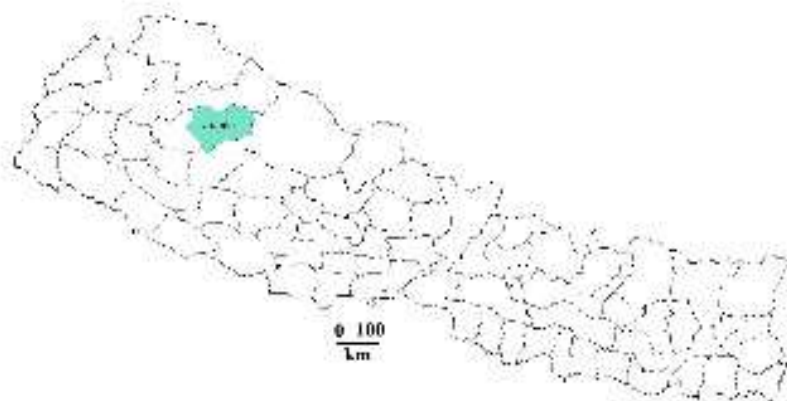


Legends

- 1 RIM-8, KHALIBAZAR, SALYAN_SAL3
- 2 RIM-8, SALYAN_SAL3
- 3 RIM-8, KHALIBAZAR, SALYAN_SAL3
- 4 DHANBAG-7 ,KAPURKOT , SALYAN_SAL3
- 5 DHANBAG-7 ,KAPURKOT, SALYAN_SAL3
- 6 DHANBAG-7 ,KAPURKOT, SALYAN_SAL3
- 7 DHANBAG-1, KHIMCHAUR ,, SALYAN_SAL3
- 8 DHANBAG-2, MULPANI ,SALYAN_SAL3
- 9 SARASWATI NAGAR PALIKA , SHREENAGAR , SALYAN_SAL3
- 10 KUPINDE-1 , JAYATPANI, SALYAN_SAL3

- ▲ River
- ▲ Contour





Legends

- 1 PATMARA-2, JUMLA_JUM2
- 2 CHANDANNATH-2, ACHARYABADA, JUMLA_JUM2
- 3 CHANDANNATH-2, THINKE, JUMLA_JUM2
- 4 PATMARA-2, RAKSHE DHUNGA, JUMLA_JUM2
- 5 DEPALGAUN, BANKABADI, JUMLA_JUM2

- River
- Contour

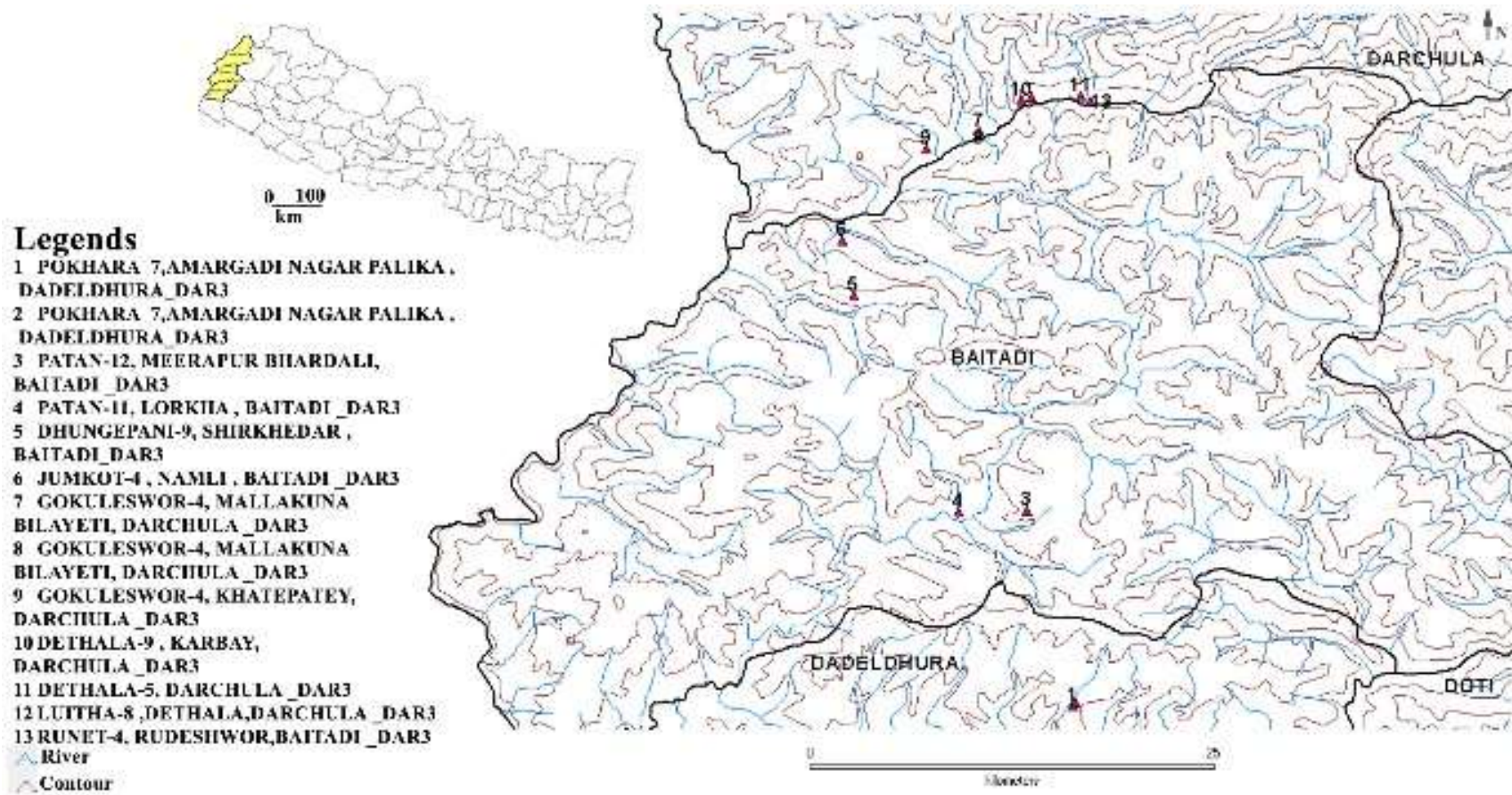


Figure 1: Maps of surveyed districts with GPS locations: Ilam, Makawanpur, Salyan, Jumla and Darchula (starting from top to down)

2.3 Orientation Training

As explicitly mentioned in the objectives of the program, orientation training entitled "*Survey surveillance: pest database preparation on five highly traded MAPs*" was organized to the concerned personnel to execute the program. An eight days long (17 to 24 March 2014) orientation training with major emphasis on pest survey surveillance as well as pest identification and preparation of pest database was provided to fifteen participants of diverse sector (Herbal company representatives, university students, concerned government staffs, field botanist, entomologists and pathologists) concerned with the program (Annex II).

2.4 Preparing host specific pest detection survey plan

NPQP took the responsibility to prepare host specific pest detection survey plan for five targeted species. NPQP referred the literatures and carried out expert group consultation and drafted the draft pest survey and surveillance guideline (Annex III to Annex VII). The guideline was duly approved by NPPO and the final version was published by NPQP in November, 2014.

2.5 Preparation of survey surveillance guidelines

A final draft of technical guideline on detection survey and surveillance of plant pests in Nepal, 2014 has been produced by National Plant Protection Organization (NPPO), a government body, focal organization for Sanitary and Phytosanitary Issues of WTO. An ownership on the document has thus been created by doing this particular activity through NPPO.

2.6 Field visit

Survey Plan: The expert team agreed to carry out the survey in at least three seasons (within a year).

First survey: During April-May, 2014 (seedling, growing and sprouting stage)

Second survey: During July-Aug, 2014 (flowering and fruiting stage)

Third survey: During Oct-Nov, 2014 (harvesting and post harvesting stage)

According to prepared detection survey plan, fifteen field visits with reference numbers were planned for sample collection which is as follows:

Table 3: List of selected districts with code numbers

Code No.	District	Sample	Seedling/Sprouting	Flowering and fruiting stage	Harvesting Stage
			April-May, 2014	July-Aug, 2014	Oct-Nov, 2014
1	Makwanpur	Wild Asparagus	Mak1*	Mak2	Mak3
2	Illam	Chiretta	Ila1	Ila2	Ila3
3	Salyan	Prickly Ash	Sal1	Sal2	Sal3
4	Darchula	Soapnut	Dar1	Dar2	Dar3
5	Jumla	Gentian	Jum1	Jum2	Jum3

* Mak1 indicates code number given to the collection site as code number of district/ place, followed by plot number, subplot number - sample number. For eg. Mak1.P2.SP1-S3 means third sample collected from first subplot of second plot of Makwanpur.

2.6.1 Composition of the survey team

The survey team members comprise of concerned experts associated with the project and other related fields. Each field visit comprises the following personnel:

1. Team leader – 1
2. Ecologist – 1
3. Plant Pathologist – 1
4. Entomologist – 1
5. Research Associate – 1

2.6.2 Sample Collection

Sampling method: Stratified as well as randomized sampling methods were applied for sample collection. Full sample observation was undertaken on all randomized sub-plots until the species accumulation curve was satisfied. Clustered locations were randomly sampled according to horizontal distribution and altitudinal gradients. Field survey work was accomplished as per the protocol mentioned in the NPPO survey guideline. Storage conditions and incidence of different pests in the storage facilities of the farmers, local road head collectors and traders were observed and studied to obtain information on the storage practices, conditions and pests. Weeds were visually observed and identified in the field using the expert's knowledge, referring to literatures and herbarium are prepared for unidentified weeds, which were later identified from the central Herbarium.

2.6.3 Basic specimen collection procedures:

- The tools were sterilized with 70% ethanol before and after each sampling.
- Soil and crown (lower stem) tissues with root samples were collected if the problem was associated with the root.
- The time was kept to a minimum between sampling and dispatch of the sample for identification.
- The methods mentioned on “Technical Guidelines for Detection Survey of Plant Pests in Nepal” published by NPPO 2014 was followed in most of the cases.

Insect samples:

A large number of specimens of all life stages were collected. The specimens were collected with appendages such as antennae, wings and legs in unbreakable form. Leak-proof alcohol and resistant container was used with screw-top lid. For small and/or soft bodied insects (example: thrips, aphids, mites, larvae, etc), 70% ethyl alcohol was used. For hard-bodied insects (example: beetles, moths, grasshoppers, fruit flies), specimen was folded in tissue paper and was kept in crush-proof plastic tube or container with several holes in the lid for ventilation.

Fungus and Bacteria:

Fungal and bacterial samples was stored under 2 - 5° C as long as possible. The samples were selected at the margin between the diseased portion of the plant and the healthy portion. Fresh, representative and generous sample covering the full range of symptoms was collected. Soil and crown (lower stem) tissues with root samples were collected if considered to be a root problem. Samples were placed in self-sealing ventilated plastic bags with some dry tissues or paper towel to absorb excess moisture.

Nematodes:

The soil for the sample was taken at least 5 - 10 cm below the surface. Individual sample size was about 250–300 g. Affected stems or leaf material was kept separate from soil and/or root samples.

Weeds:

Associated weeds were identified by the expert’s knowledge and with the help of relevant literatures. Unidentified weeds were preserved in a herbarium sheet (ca. 450 × 300 mm).

2.6.4 Preparation of voucher specimens of selected MAPs and accompanying pests

Voucher specimens of insect samples collected were first killed in carbon killing agent (carbon tetra chloride) then preserved in 70% ethyl alcohol. Larva samples were treated with hot water and then preserved in 70% alcohol. Diseased samples were dried and preserved in pressed form. All the samples were stored in DPR microbiology laboratory following specific code numbers for identification process.

Interpretation of code number of samples:

Code number assigned to the isolated pests of particular plant consists of seven letters. First two letters are the first letter (in capital) of generic name and first letter of species name of plant from which the pathogen is isolated. Third (in capital), fourth and fifth letters shall be the first three letters of place/district followed by (#) field visit number. Sixth (*) letter shall be the digit that indicates the serial number of collected sample. Last letter shall be the first two letter of part of plant. For eg, Sc-Ila1-2-Le indicates pathogen isolated from second sample of leaf of Chireeta collected from Ilam.

Table 4: List of Code numbers assigned to the isolates

District	Sample	Leaf	Stem	Root	Fruit	Seed
Makwanpur	Wild Asparagus	Ar-Mak#- *-Le	Ar-Mak#- *-St	Ar-Mak#- *-Ro	Ar-Mak#- *-Fr	Ar-Mak#- *-Se
Ilam	Chireeta	Sc-Ila#-*- Le	Sc-Ila#-*- St	Sc-Ila#-*- Ro	Sc-Ila#-*- Fr	Sc-Ila#-*- Se
Salyan	Prickly Ash	Za-Sa#-*- Le	Za-Sal#- *-St	Za-Sal#- *-Ro	Za-Sal#- *-Fr	Za-Sal#- *-Se
Darchula	Soapnut	Sm-Dar#- *-Le	Sm-Dar#- *-St	Sm-Dar#- *-Ro	Sm-Dar#- *-Fr	Sm-Dar#- *-Se
Jumla	Gentian	Ns-Jum#- *-Le	Ns-Jum#- *-St	Ns-Jum#- *-Ro	Ns-Jum#- *-Fr	Ns-Jum#- *-Se

2.7 Laboratory analysis and identification of organisms

The processing of the field collected samples and identification was done at biology section of DPR. Standard procedures were followed for laboratory analysis of the samples. As per the types of samples collected, following methods were applied,

- Mycological Method
- Bacteriological Method
- Entomological Method
- Weeds

2.7.1 Mycological Method

2.7.1.1 Isolation of organisms

Isolation from soil

Dilution plate technique was applied to study the soil flora in rhizosphere soil. Soil samples were air-dried without severely desiccating them. A small amount of the sample was grinded in a sterilized mortar to break the lumps. Large particles were removed and 10 gm of the grinded sample was weighed and taken in a 100 ml conical flask. Sterilized water was added making the total volume to 100 ml and the mixture was shaken vigorously for ten minutes. One ml of this stock solution was added to another test tube containing 9 ml sterilized water to prepare the suspension of 10^{-1} strength. Similarly the suspensions of strengths 10^{-2} , 10^{-3} and 10^{-4} were prepared by serial dilution. For the study of soil flora, two types of media were used viz. PDA (Potato Dextrose Agar) and TWA (Tap Water Agar). The poured plates were allowed to dry in cool, dark place for 3-5 days before use. One ml of the 10^{-1} solution was poured on the surface of the dried media and was spread over the agar surface using a sterilized glass spreader. This process was repeated for each strength (except 10^0) and two replicates were prepared for each strength. The petridishes were incubated at $25\pm 2^\circ\text{C}$. The petriplates showed microbial growth from second day of inoculation. The culture plates were examined from second to fifth day. The colonies were counted on the fifth day and the organisms were examined, identified and transferred to fresh PDA plates for further study.

Isolation from plant materials

The infected samples via root, stem, leaf, fruit and seed were cut into 3-5mm pieces and were washed well in sterilized water to remove adhering soil particles. These were then dipped in 75% alcohol to make them readily extractable. The pieces were immersed in 0.35% of sodium hypochlorite solution for 1 minute. They were then rinsed for three times thoroughly with sterilized water. The pieces were blotted with sterilized blotting paper to remove excess water. The ends of the pieces were cut off with a sterilized scalpel and central parts were plated out. The pieces thus sterilized were used for isolation by following procedures.

Moist blotter method

Moist chambers were prepared by placing three layers of sterilized blotting papers moistened with sterilized water on the bottom of petridishes. The four sterilized pieces of plant parts were placed

on the blotting paper and 25 seeds of Prickly ash per plate with four replicates of each sample were inoculated. The plates were incubated at $25\pm 2^{\circ}\text{C}$. The observations were made from second to seventh day. The fungi growing on the pieces were examined and identified. The number of pieces showing fungal growth was noted on the seventh day. The fungi were then transferred to PDA and TWA

Agar plate method

The sterilized pieces were blotted with sterilized blotting paper to remove excess water. The four sterilized plant parts were then plated on PDA and TWA. Four replicates were maintained for each sample. The plates were then incubated at $25\pm 2^{\circ}\text{C}$. These showed fungal growth from second day onwards and the observations were made from second to fifth day. The number of pieces showing fungal growth was counted and the fungi were transferred to PDA plates for further study (Booth, 1971).

2.7.1.2 Single spore culture of isolated species

The species isolated from various plant parts were further purified by single spore culture. A needle, sterilized by flaming, was introduced into a well-grown colony of fungi (7 days old) so that spores adhered to it. Spores adhering to the needle-tip were dusted onto the surface of a TWA plate. With the help of a microscope, a consolidated spore was located and was transferred along with some agar to a fresh PDA plate with the help of a sterilized needle. The plates were then incubated at $25\pm 2^{\circ}\text{C}$ (Booth, 1971).

2.7.1.3 Identification of organisms

Preliminary identifications were done on the basis of habit characters *in situ*, direct observation under microscope and by microscopic observation of prepared slides.

Determination of growth rates and pigmentation was done following Booth (1971 c and 1977). Maximum and minimum diameters of seven-day-old single-spore-culture colony of an isolate were measured and average colony diameter for each isolate was calculated. For the observation of the pigmentation, the cultures were exposed to normal daylight for four days, and the colour was determined visually.

The identification of the isolated fungus were done by following the methods given by Barnett, H.L. (1960); Booth, C. (1971 and 1977); Gilman, J.C. (1957); Neergaard, P. (1979); Mathur, S.B.

and Kongsdal, O. (2003); Cappuccino, J.G. and Sherman, N.(1999); FAO Manual (1992) and Bergey's Manual of Systematic Bacteriology, Vol. I (1984).

2.7.2 Bacteriological Method

2.7.2.1 Isolation and purification of bacterial isolates

Isolation and purification was done following FAO Manual (1992). Suspected bacterial colony isolated from different affected plant parts were streak on prepared Nutrient Agar plates (NA) by streak plate method. The NA plates were incubated for 24 to 48 hrs at 37°C and subsequently observed for bacterial growth and colony morphology. After isolation, the isolates were purified to single colony for identification. Then using sterile inoculating technique, inoculate two Nutrient Agar slants by means of a streak inoculation and all cultures were maintained at 4°C.

2.7.2.2 Identification of Bacteria

All isolated cultures were identified by different test according to Bergey's Manual of systematic bacteriology (1984) as gram stain for morphology and arrangement of cell and inoculate in different media following for biochemical tests.

The principal tests used for this purpose are Carbohydrate Fermentation Test, Indole Test (IND), Methyl Red Test (MR), Voges-Proskauer Test (VP), Citrate Utilization Test (CIT), Urease Test (URE), Nitrate Reduction Test (NIT), Oxidase Test (OXI), Catalase Test (CAT), Hydrogen Sulphide Production (H₂S), Starch Hydrolysis, lipid hydrolysis, gelatin liquefaction Aerobic and Anaerobic Test (Ae/An). For all tests the cultures were inoculated and incubated at 37°C for 24 to 48 hrs. After incubation, a positive result was noted as change of color to yellow, while no color change was observed in negative results. IND test was performed by culturing the micro-organisms in peptone water medium containing tryptophan in a screw capped tube, incubated for 24 hr at 37°C and then Kovac's reagent (0.5 ml) was added where the positive results were indicated by the formation of pink red layer on the broth within seconds of adding Kovac's reagent. MR test was performed by inoculation of the glucose phosphate peptone water in a screw capped tube, incubation for 24-48 hr and then addition of 5 drops of methyl red where the change in color of the medium to cherry red was considered as positive. VP test was performed by inoculating glucose phosphate peptone water with the microbial isolates in a screw capped tube, incubating for 24-48 hr, then adding of 0.6 ml of alpha-naphthol solution and 0.2 ml of Potassium Hydroxide solution.

The tubes were then allowed to stand for 5-10 min after shaking well. The red color formation was taken as the positive result. For the CIT test, the Simons citrate agar slants were inoculated and incubated at 37°C for 24-48 hrs. The positive slants were noted to change color from green to blue. For URE test, urea broth was inoculated and incubated at 37°C for 24 to 48 hrs. The change of color of the broth from yellow-orange to bright pink was considered as positive.

For NIT test, nitrate broth was inoculated and incubated at 37°C for 24-48 hr. After incubation, 5 drops of Sulfanilic acid and 5 drops of N-dimethyl-1-naphthylamine were added. The change of color of broth to deep red within 5 min. meant that the bacteria had produced nitrate reductase. If color did not change, the results were indecisive. Small amount of zinc was added to the broth. If the solution remained colorless, then both nitrate reductase and nitrite reductase were present. If the solution turned red, nitrate reductase was not present.

OXI test was used to assess the bacteria which produce the enzyme cytochrome oxidase. Filter paper was moistened with a few drops of 1% Tetramethyl-p-phenylenediaminedihydrochloride. With a wooden applicator, growth from TSA plate was smeared on the paper. A positive result was the development of purple color. No color change indicated a negative result. CA test was performed by adding a small amount of bacterial isolate into freshly prepared 1% hydrogen peroxide, and the bubbles of oxygen if appeared the isolate was considered as positive for CA test. For Ae/An test, TSA was inoculated and incubated at 37°C in anaerobic jar. The growth was observed after 24-48 hrs.

Hydrogen Sulphide test (H₂S) and Motility test was used to determine the ability of an organism to reduce sulphur into H₂S and motile and non-motile respectively. SIM media was used for the H₂S production and motility test. SIM media contains the sulfur containing amino acid, sodium thiosulfate, cysteine and ferrous sulfate. The SIM media was inoculated with bacterial cultures by stabbing SIM media with inoculating needle. The tubes were then incubated at 35°C for 24 hr. After incubation, a positive result was indicated by a black precipitate formed because of the reaction of H₂S with the iron or ferrous sulfate; while the negative result was indicated by no black precipitate. All results were record in chart and identify to genus and species of the unknown organisms comparing with reference cultures.

2.7.3 Entomological Method

The specimen processing and identification was done following the method mentioned at the “Technical Guidelines for Detection Survey of Plant Pests in Nepal” (NPPO, 2014). The collected insect specimens were processed according to the insect orders. The insect specimens collected from the field were transported as wet (in 70 percent alcohol) or dry transported methods. These specimens were placed at DPR laboratory following temporary preservation methods for some while and further preservation was done as soon as possible. The small and soft bodied insects, kept at 70% alcohol, were processed at first by changing the alcohol content and the insects were kept at screw capped vials. Thereafter these specimens were cautiously taken out from the vial using standard camel hair brush and identified using stereo microscope.

The dry transported specimens to DPR laboratory were placed at white paper and sorted out according to the insect orders. Then all of these insect specimens were kept at relaxing jar for more than 24 hrs. These specimens were then pinned with standard entomological pin following standard pinning methods. Later these insects were identified based on morphological characteristics using stereo microscope. The identification of both wet and dry preserved insect specimens was followed as mentioned in IMMS taxonomy, Dulong and Borer, CPC. Further, the information made available by accredited institutions via internet sites was also referred.

2.7.4 Weed Method

Each survey team composed of a botanist and s/he identified most of the weeds in the field with the help of own experience and by referring to different literatures like Polunin and Stainton, 1984; Stainton 1988; Press *et. al*, 2000 etc. Herbarium were prepared for the unidentified ones and were identified cross matching with the herbarium housed in National Herbarium and Plant Laboratory, Godawari.

CHAPTER III: FINDINGS

The basic information regarding outcome from field survey and laboratory analysis are mentioned in this chapter. The information of plant diseases, insects and weeds are presented separately for each surveyed MAP commodities. The consecutive field visits with their code numbers are arranged in each commodities finding. Also, some notes on field observation are also included.

3.1 Findings of pest survey in Wild Asparagus

First Field: Vegetative stage

1. Mak1.P1.SP1 : Kamane, Hetauda Municipality- 8
2. Mak1.P1.SP2 : Kamane, Hetauda Municipality- 8
3. Mak1. P2 : Gutrum Danda, Dallapata, East, Terrace
4. Mak1.P3 : Jyamire, Phaper Bari VDC
5. Mak1.P4 : Prem Nursery, Manahari VDC

Second Field: Flowering and fruiting stage

1. Mak2.P1.SP1 : Thulogangate, Hatiya VDC
2. Mak2.P1.SP2 : Thulogangate, Hatiya VDC
3. Mak2.P1.SP3 : Thulogangate, Hatiya VDC
4. Mak2P2.SP1 : Badkaule, Harnamandi VDC
5. Mak2P2.SP2 : Badkaule, Harnamandi VDC
6. Mak2P3.SP1 : Prem Nursery, Manahari VDC
7. Mak2P3SP2 : Prem Nursery, Manahari VDC

Third Field: Harvesting and storage

1. Mak3.P1 : Badkaule, Harnamadi VDC, North, Terrace
2. Mak3.P2 : Kamane, Hetauda Municipality-8, North
3. Mak3.P3 : Prem Nursery, Manahari VDC

The pest collected and identified and their symptomatic characteristics are given below

3.1.1 Plant Pathological Findings

Alternaria alternata (Fr.) Keissl. (Pleosporales: Pleosporaceae)

Sample number	Mak1.P1.SP1-S5
Isolate number	Ar-Mak1-1-Le

The disease specimen was collected from the leaf of the plant (Mak1.P1.SP1) (Fig. 2). The brown spots at the leaf were observed with tip drying of affected leaves. The further field information of *A. alternata* in Wild Asparagus plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.2 cm. Colony appear circular in form, entire margin with raised elevation, powdery to felty. The colony appear grey to olivaceous green with white margin in obverse and appear dark brown black with light brown periphery.

Morphological characteristics:

Mycelium sparse, greyish brown, fluffy septate hyphae; conidia variable in shape often ellipsoidal, obclavate or ovoid, pale to dark brown with 3-7 transverse septa and 1-several longitudinal septaidium 28-58×10-25 µm. Conidium is polymorphous formed in branching chains (Plate 1).



Figure 2: Brown spots on leaf of Wild Asparagus plant

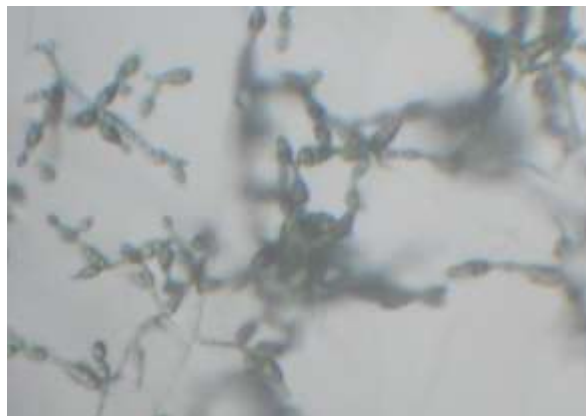


Plate 1: Microscopic photo of *A. alternata* of Wild Asparagus

Chaetomium sp. (Sordariales: Chaetomiaceae)

Sample number	Isolate number
Mak1.P1-S5	Ar-Mak1-2-Le
Mak1.P1 -S7	Ar-Mak1-3-Le
Mak1.P1 -S7	Ar-Mak1-4-St
Mak1.P1 -S9	Ar-Mak1-5-St
Mak1.P1 -S3	Ar-Mak1-6-St

The disease specimens were collected from the leaf of the plant (Fig. 3) from Mak1.P1.SP1 .The black shooty symptoms with brown concentric spindle shaped lesion, unilateral browning and drying at the stem and unilateral browning with tip die back symptom of leaf were observed.

The further field information of *Chaetomium* sp. in Wild Asparagus plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungi is 6 cm. The colony consist irregular margin with flat elevation. The colony consists of white ridges with dots at obverse and yellow ridges at reverse.

Morphological characteristics:

The fungus produces conglomerate mycelium with lemon shape ascospore in globose black ascus(Plate 2).



Figure 3: Unilateral browning on stem of Wild Asparagus plant

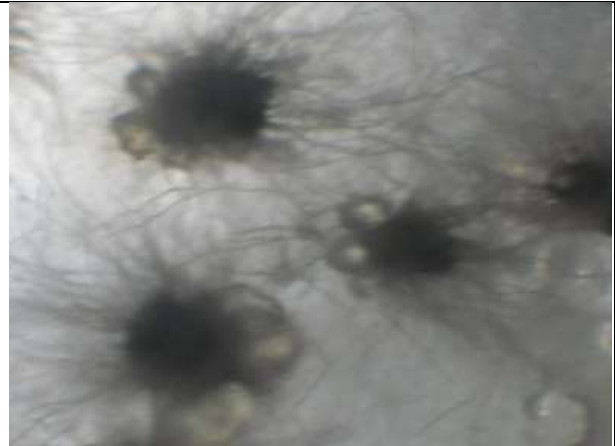


Plate 2: Microscopic view of *Chaetomium sp.*

Cladosporium sp. (Capnodiales: Davidiallaceae)

Sample Number:

Mak1.P3.S-18

Isolate number:

Ar-Mak1 -7-St

The disease specimens were collected from the stem of the plant (Fig. 4) from Mak1.P3. The symptom of die back was observed at stem. The further field information of *Cladosporium sp.* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 2.1 cm. The colony consists irregular in form, compact and with raised elevation. The colony of fungus at obverse appears army green and black at reverse.

Morphological characteristics:

The fungus produces aerial mycelium with coiled hyphae. The conidia appear in 3-5 chain, shape ranges from ovate to cylindrical with olive brown color. The size of conidia ranges 3-4 μm .



Fig 4: Die-back symptom of Wild Asparagus plant



Plate: Conidia of *Cladosporium* sp.

***Pectobacterium carotovorum* (Jones) Waldee (Enterobacteriales: Enterobacteriaceae)**

Sample number	Isolate number
Mak1.P1.S7	Ar-Mak1-8-Le
Mak1.P4-S3	Ar-Mak1-9-St
Mak3.P3-S1	Ar-Mak3-10-Ro

The disease specimens were collected from the leaf, stem and root of the plant (Fig. 5) from Mak1.P1, Mak1.P4 and Mak3.P3. The brown lesion of stem and root rot was observed. The pathogen (Plate 4) was isolated from leaf during first visit (Fig. 5) and from rhizome on third visit. The further field information of *Pectobacterium caratovora* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The fungus has mucoid growth which is white and moist.

Morphological characteristics

The vegetative cell of bacterium is straight, long and rod shape. The bacterium shows Gram Negative in Gram staining (Plate 4).



Fig 5. Brown lesions on leaf and stem of Wild Asparagus plant.



Plate 4: Microscopic view of *Pectobacterium caratovora*

***Fusarium oxysporum* Schlecht. (Hypocreales: Nectriaceae)**

Sample number	Isolate number
Mak1.P3.S-9	Ar-Mak1-11-St
Mak3.P2.S-1	Ar-Mak3-12-Ro

The disease specimens were collected from the stem of the plant (Fig. 6) from Mak1.P3 and Mak3.P2. in the storage . The unilateral browning of stem and white small powdery spots on root were observed. The pathogen (Plate 5) was isolated from stem during first visit and from rhizome (Fig. 7) on third visit. The further field information of *F. oxysporum* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The growth rate of fungus is 4.5 cm. Its colony is circular, floccose, crateriform in elevation with entire margin. The obverse and reverse view of fungus is pinkish white to purple.

Morphological characteristics

The fungus produces aerial, septate and branched mycelium. The fungus micro conidia is oval to ellipsoidal which size ranges 5-12×2.5-3.5 μm while macro conidia is fusiform that is slightly curved with 3-5 septa that size ranges 24-45×3-5 μm (Plate 5).



Fig 6. Browning of stem



Plate 5. Microscopic view of *F. oxysporum*



Fig 7. White powdery spot and root rot

***Fusarium solani* (Mart) Sacc. (Hypocreales: Nectriaceae)**

Sample Number	Isolate number
Mak1.P5.S-1	Ar-Mak1-13-St
Mak3.P1.S-1	Ar-Mak3-14-Ro

The disease specimens were collected from the stem of the plant (Fig. 8) from Mak1.P5 and root of the plant from Mak3.P1. (Fig 9). The brown spots of stem and root rot was observed. The further field information of *F. solani* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The growth rate of fungus is 3.2 cm. The colony of fungus is regular, floccose with raised elevation. At obverse the fungus is white to cream while reverse view is yellowish with white periphery.

Morphological characteristics

The mycelium is hyaline, aerial and septate. The microconidia is cylindrical to oval which size ranges 8-15x2-4 μm and macro conidia is inequilaterally fusoid with 1-5 septa which size ranges 35-55x4.6 -6 μm (Plate 6).



Fig 8. Brown spots on stem of Wild Asparagus plants



Plate 6. Microscopic view of *Fusarium solani*.



Fig 9. Brown spots on root of Wild Asparagus plant.

***Bipolaris sorokiniana* (Sacc) Shoem. (Pleosporales: Pleosporaceae)**

Sample number

Mak1.P4.S3

Isolate number

Ar-Mak1-15-St

The disease specimens were collected from the stem of the plant (Fig. 10) from Mak1.P4. The brown spots of stem were observed. The further field information of *B. sorokiniana* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The growth of the fungus is 4.5 cm. Its colony is irregular and flat. The obverse view of fungus is grayish black while reverse view is grayish black with dark brown ridges.

Morphological characteristics

The fungus produces septate and hyaline mycelium which bears unbranched conidiophores. The conidia is ellipsoid, dark brown, smooth walled with ends rounded which is broad at middle. It bears 3-10 distosepta, which size ranges 40-100×17- 23 μm (Plate 7).



Fig .10. Brown spots on stem of Wild Asparagus plant.



Plate 7. Microscopic view of *Bipolaris sorokiniana*

Fusarium moniliforme Sheldon (Hypocreales: Nectriaceae)

Sample number	Isolate number
Mak2.P1.S-2	Ar-Mak2-16-St
Mak2.P2.S-1	Ar-Mak2-17-St

The disease specimens were collected from the stem of the plant (Fig. 11) from Mak2.P1 and Mak2.P2. The brown spots of stem were observed. The further field information of *F. moniliforme* in Wild Asparagus plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics

The growth rate of fungus is 4.5 cm. The colony of fungus appears floccose which is circular in form. At obverse view of fungus is white while at reverse view of fungus is peach centre with white periphery.

Morphological characteristics

The fungus produces hyaline, septate, sparsely branched mycelium. The micro conidia are hyaline, clavate bearing in long chain which size ranges 6-10x2-2.5 μm . The macro conidia are fusoid with 3-6 septa which size ranges 25-35x 2.5-4 μm (Plate 8).



Fig. 11. Brown spot on the stem of the Wild
Asparagus Plant



Plate 8. Microscopic view of *Fusarium moniliforme*

***Fusarium poae* (Peck) Wollenw. (Hypocreales: Nectriaceae)**

Sample number	Isolate number
Mak2.P1.S4	Ar-Mak2-18-St

The disease specimens were collected from the stem of the plant (Fig. 12) from Mak2.P1. The unilateral browning of stem was observed. The further field information of *F. poae* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The growth rate of fungus is 7.6 cm. Its colony appears floccose with irregular form. The colony is white to yellow in obverse view where as peach centre with dull white periphery at reverse view.

Morphological characteristics

The fungus consist hyaline, aerial and septate mycelium. Its macro conidia are fusiform with 1-3 septa that measures 20-30x2-3 μm . Its micro conidia are ampulliform to globose, aspetate measuring 7-10 μm (Plate 9).



Fig 12: The Unilateral browning of stem

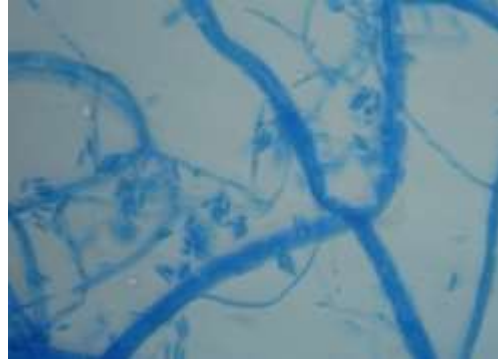


Plate 9: .Microscopic view of *Fusarium poae*.

***Alternaria radicina* Meier, Drechsler & E.D. Eddy (Pleosporales: Pleosporaceae)**

Sample number
Mak2.P1.S4

Isolate number
Ar-Mak2-19-St

The disease specimens were collected from the stem of the plant (Fig. 13) from Mak2.P1. The unilateral drying of stem was observed. The further field information of *A. radicina* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The growth of fungus is 4.3 cm. The colony appears circular in form, flat elevation with entire margin. The colony of fungus appears brownish green at obverse and dark brown with light periphery at reverse.

Morphological characteristics

The fungus produces grayish, fluffy mycelium in which black shiny conidia appears single or in pairs arranged in short chains. The conidia are variable in shape which is often ellipsoidal or may be obclavate or ovoid. Color of conidia is pale to dark brown with 3-7 transverse septa and one or several longitudinal or oblique septa which size is 27-57x9-27 μm (Plate10).



Fig 13: Unilateral drying of the stem of the Wild Asparagus plant

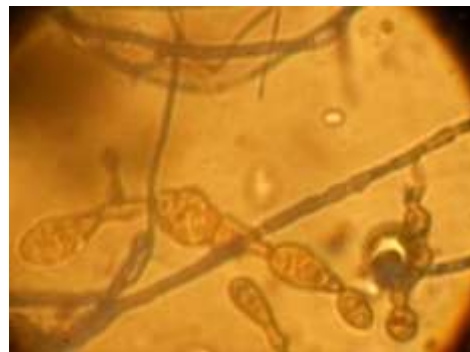


Plate 10. Microscopic view of *A. radicina*

Colletotrichum gloeosporiodes Dickman (Glomerellales: Glomerellaceae)

Sample number	Isolate number
Mak2.P1.S4	Ar-Mak2-20-St

The disease specimens were collected from the stem of the plant (Fig. 14) from Mak2.P1. The unilateral drying of stem was observed. The further field information of *C. gloeosporiodes* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 2 cm. The fungus colony is floccose, loose and filamentous. The colony appears dull white at obverse and orange with white periphery at reverse.

Morphological characteristics:

The fungus produces hyaline, white and shiny mycelium. The conidia are non-septate, cylindrical with ends rounded. The size of conidia measures 6-14x3-4 μm (Plate 11).



Fig 14: Unilateral drying of the stem of the Wild Asparagus Plant

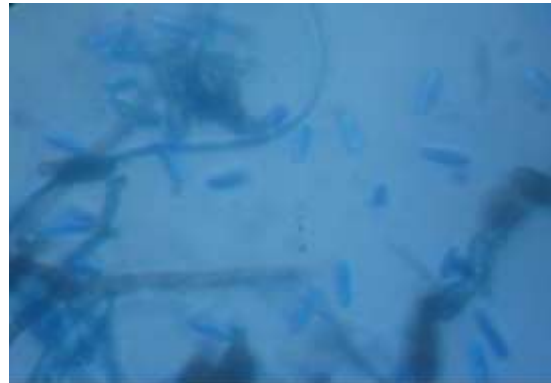


Plate 11: Microscopic view of *C.gleospodiodes*

Fusarium dimerum Penzig (Hypocreales: Nectriaceae)

Sample number	Isolate number
Mak2.P3.SP2	Ar-Mak2-21-Ro

The disease specimens were collected from the root of the plant (Fig. 15) from Mak2.P3.SP2. The tip die back of the plant was observed. The further field information of *F. dimerum* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of the fungus is 2.7 cm. The colony is floccose, circular in form, umbonate in elevation with entire margin. The fungus on obverse view appear white while in reverse appear light orange.

Morphological characteristics:

The fungus produces hyaline and septate mycelium. Its macro conidia is fusiform that is strongly curved with pointed apex. The fungus consists of 1-3 septa. Its size varies 10-20x3-4 μm . Micro conidia is absent (Plate 12).



Fig 15: Tip down die back of Wild Asparagus Plant



Plate 12. Microscopic view of *F. dimerum*

Geotrichum candidum Link (Saccharomycetales: Endomycetaceae)

Sample number: Mak3.P2.S1

Isolated number: Ar-Mak3-22-Ro

The disease specimens were collected from the root of the plant (Fig. 16) from storage of Mak3.P2. The small white powdery spot on root was observed. The further field information of *G. candidum* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The growth rate of fungus is 4 cm. The colony appears circular with entire margin, flat elevation with characteristic smell. The colony is white at obverse and creamy white at reverse.

Morphological characteristics

The fungus mycelium is hyaline, septate and branched. The conidia are hyaline, single celled, short and cylindrical with truncate ends (Plate 13).



Fig 16: Small powdery spots on the root of plant

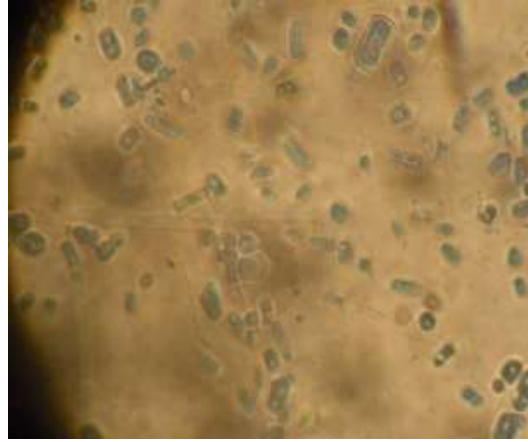


Plate 13. Microscopic view of *Geotrichum candidum*

***Colletotrichum lindemuthianum* Sacc. & Magnus, *Michelia* (Sordariomycetidae: Glomerellaceae)**

Sample number: Mak3.P3-S2

Isolate number: Ar-Mak3-23-St

The disease specimens were collected from the stem of the plant (Fig. 17) from Mak3.P3. The brown spot on the stem of the plant was observed. The further field information of *C. lindemuthianum* in Wild Asparagus plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The colony is irregular, cottony and floccose. The colony at obverse appears gray centre with white periphery and while in reverse appears grayish black centre with white periphery.

Morphological characteristics

The mycelium is hyaline and consist septa. The colony is hyaline, oblong to dumbbell shaped, 1-celled with ends rounded which measures 9-14x3-4 μm (Plate 14).



Fig 17: Brown spot on the stem of Wild Asparagus Plant



Plate 14: Microscopic view of *C. lindemuthianum*

3.1.2 Entomological Findings

Horned coreid bug, *Cletus* sp. (Hemiptera: Heteroptera: Coreidae)

General introduction of insects:

The horned coreid bug, *Cletus* sp. was observed during first (Mak1.P4) and second visit (Mak2.P3.SP1 and Mak2.P3.SP2) at Wild Asparagus plants. This insect has been reported especially from oriental region from different habitat. This bug was collected on the foilage and fruits of plants at the surveyed areas (Fig. 18). It has been reported that the bug suck the skin of immature fruit thereby influencing the seed quality. However the insect has been reported as of minor importance to the associated plants. The bug is medium sized stout brown colored with the body length about 7 mm in length (Plate 15). Although the bug remembles with *Cletus punctulatus* Westwood, but it needs further confirmation. The field information of horned coreid bug in wild asparagus plant is described in pest data sheet (Annex XII).

The bugs were collected with the help of sweep net from the plant canopy. After collection, the insect was kept at the killing jar for some time and later transferred to 70% alcohol solution in 50 ml plastic bottle. Later this insect was placed at 30 ml screw capped vial filled with 70% alcohol and transported to the DPR laboratory.

At the laboratory, the specimen was taken out and the alcohol content was dried using blotting papers. Then the stink bug was pinned at the left sculletum by standard insect pin and kept fom three days for completely drying. After, the dried and pinned specimen was kept at the standard insect box at DPR laboratory.



Fig 18: Horned coreid bug at the foilage of wild asparagus plant Plate 15: Horned coreid bug

Wild asparagus beetle: *Crioceris* sp.; Coleoptera: Chrysomelidae

General introduction of insects:

The chrysomelidae beetle was found from almost all the surveyed sites during first and second visit at wild asparagus plants. The asparagus beetle we observed at the wild asparagus field is different from other two chrysomelidae beetels (*Crioceris asparagi* (L.) and *Crioceris duodecimpunctata* (L.)) reported from garden asparagus (Hexamer, 2010) and another chrysomelidae beetle (*Lema downesi* Baly) reported from wild asparagus (ICAR, 2009). Going through the literatures, the beetle remembles with *Crioceris bicrucata* Sahlberg, but it needs further confirmation. Although the damaging behaviour seems to be similar with other beetles, but taxonomically it is different. So, this beetle may be host-specific to wild asparagus plants and needs further detail research on other aspects. At the field the beetles were found in large number damaging the tender leaves and shoots, so it is economically very important species for wild asparagus. The field information of chrysomelidae beetle in wild asparagus plant is described in pest data sheet (Annex XII).

Visible damage symptoms in commodity pathway:

Both adult and the grub are damaging stages. As the wild asparagus plant is bushy in nature, the adult and larvae hide under the bushes and damage the plant. As of other chrysomelidae beetles, the asparagus beetle also possess habit of falling down and feign death character when distured. Similarly the larvae with its viscous secretions attached firmly at the tender shoot. The grubs, as well as the beetles, attack the tender portions of the plants, but in many cases the grubs gnaw the

epidermis of the stems. The beetles also gnaw the young shoots beneath the surface, causing them to become woody and crooked in growth. During the second field visit (vegetative and flowering stage of the plant), the population of adults (15 to 20 per plant) and grubs (20 to 25 per plat) were observed more at the plants. Due to the severe beetle damage, the plants were totally defoliated and skeletonized at site number (Figure 19) of Harnamadi VDC.

Field identification of insects:

The asparagus beetle is a member of leaf beetle of the family chrysomelidae and order coleoptera. Both the larvae and adult have characteristic chrysomelidae character. The body of the adult beetle is 5 to 7 mm long with shining color (Figure 20). It has characteristic dark orange body color and each elytron is marked with three black dots. The elytra cover the whole body of the beetle and there is black longitudinal marking at the center of both elytrons. The thorax is dark reddish-orange color without any markings. The head part and underside of the abdomen is black color. The larvae are soft and fleshy with wrinkled body pattern (Figure 21). It is about 7 to 8 mm long with dark cream colored body and black head part.

Collection, transportation and preservation of the specimen:

The adult beetle due to its active behaviour is hard to collect but larvae were easily collected as they remain attached with the shoots. The adults were collected using the sweep net and later on kept at killing jar. These adults were then transferred at 30 ml screw capped vial filled with 70% alcohol and transported to the DPR laboratory. At the laboratory, the specimen (Plate 17) was taken out and the alcohol content was dried using blotting papers. The adult beetles were pinned at the left elytra by standard insect pin and kept for three days for completely drying. After, the dried and pinned specimen was kept at the standard insect box at DPR laboratory.

The larvae at the field were collected using fine camel brush. The 30 ml screw capped was placed just beneath the larvae and with gentle move the larvae are placed inside the vial. The vials with number of larvae afterwards were filled with 70% alcohol. These vials were further wrapped by cotton roll and tightly placed inside the plastic bottle and transported to the DPR laboratory.



Fig 19: Defoliated wild asparagus plant due to damage by asparagus beetle



Fig 20: Adult beetle at the foilage of wild asparagus plant



Fig 21: Larvae at the foilage of wild asparagus plant



Plate 16: Adult wild asparagus beetle

3.1.3 Weed Findings

Common tropical, sub tropical crop weeds are found associated with Asparagus. They are listed below:

1. *Ageratum conyzoides* Linn
2. *Stellaria media* (L.) Vill.
3. *Drymeria chordata* (L.) Willd. Ex Roemel & Schultes
4. *Lantana camera* L.
5. *Blumea lacera* (Burm.F.) DC
6. *Pogostemon* sp
7. *Trifolium repens* L.
8. *Viola* sp
9. *Emilia sonchifolia* (L.) DC ex Wight
10. *Mimosa pudica* L.
11. *Cyperus difformis* L.
12. *Persicaria* sp
13. *Adenophora glanduliflora* Nakai
14. *Tridax procumbens* (L.) L.

15. *Bidens pilosa* L.
16. *Cynoglossum* sp
17. *Cynodon dactylon* (L.) Pers.
18. *Clerodendron* sp

Inter-cropping or crop rotation with maize, wheat field, vegetable crop like peas, bell plant etc also practiced.

Table 5: Pest list of cultivated Wild Asparagus, *Asparagus racemosus* Willd., 2014/15

S.N.	Pest Category	Scientific Name of Pest	Common Name of Pest	Plant Parts Affected	Location
1	Fungi	<i>Alternaria alternata</i> (Fr.) Keissl.	Leaf spot	Leaf	Kamane, Makwanpur
2	Fungi	<i>Chaetomium</i> sp.	Stem rot	Leaf, Stem	Kamane, Hernamadi
3	Fungi	<i>Cladosporium</i> sp.	-----	Stem	Prem nursery, Manahari
4	Bacteria	<i>Pectobacterium caratovorun</i>	Leaf, stem and root rot	Leaf, Stem, Root	Kamane, Prem nursery
5	Fungi	<i>Fusarium oxysporum</i> Schlecht.	Rot	Stem, Root	Gutrum Danda, Sano pokhara
6	Fungi	<i>Fusarium solani</i> (Mart.) Sacc.	Rot	Stem, Root	Prem nursery, Henamadi
7	Fungi	<i>Bipolaris sorokiniana</i> , (Sacc.) Shoem		Stem	Prem nursery
8	Fungi	<i>Fusarium moniliforme</i> Sheldon	Wilting	Stem	Thulogangato
9	Fungi	<i>Fusarium poae</i> (Peck) Wollenw		Stem	Thulogangato
10	Fungi	<i>Alternaria radicina</i> Meier, Drechsler & E.D. Eddy	Black rot	Stem	Hernamandi
11	Fungi	<i>Colletotrichum gloeosporiodes</i> Dickman	Anthracnose	Stem	Hernamandi
12	Fungi	<i>Colletotrichum lindemuthianum</i> Sacc & Magnus, Michelia	Anthracnose	Stem	Prem nursery
13	Fungi	<i>Fusarium dimerum</i> Penzig	Wilting	Root	Prem nursery

14	Fungi	<i>Geotrichum candidum</i> Link	Rot	Stem,	Sano Pokhara
15	Insects	<i>Cletus</i> sp.	Horned coreid bug		
16	Insect	<i>Cricoceris</i> sp.	Wild asparagus beetle		

3.2 Findings of pest survey in Gentian plant

The pest detection survey on *Neopicrorhiza scrophulariflora* (Gentian) was conducted at Jumla district. During the first field visit, site selection work was done discussing with concerned stakeholders (DFO, DPRO, local traders, farmers). For this purposes, the procedure mentioned at “survey surveillance guidelines” published by NPPO was followed. With all these background and discussions, the following fields were identified for the detection survey.

First Field:

1. Jum1.P1.SP1 : Depalgaun, Jumla
2. Jum1.P2 : Patamara-2, Rakshedhunga, Jumla
3. Jum1.P3 : Patmara, Jumla
4. Jum1.P4 : Kale Lekh, Jumla

Second Field:

1. Jum2.P1.SP1 : Depalgaun, Jumla
2. Jum2.P2 : Patmara-2, Rakshedhunga, Jumla
3. Jum2.P3 : Patmara, Jumla

3.2.1 Pathological Findings

Geotrichum candidum Link. (Saccharomycetales: Dipodascaceae)

Sample No. : Jum1.P2-S1 Sample Number: Jum2.P3-S5

Isolate No.: Ns-Jum1-1-Le Isolate number: Ns-Jum2-2-Le

The disease specimen was collected from the leaf of the Gentian plant (Fig. 22) from Jum1.P2. and Jum2.P3. The reddish black necrotic spot and leaf spot and leaf blight near apex at the leaf were observed. The further field information of *G. candidum* in Gentian plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The growth rate of fungus is 4 cm. The colony appears circular with entire margin, flat elevation with characteristic smell. The colony is white at obverse and creamy white at reverse.

Morphological characteristics

The fungus mycelium is hyaline, septate and branched. The conidia are hyaline, single celled, short and cylindrical with truncate ends (Plate 17).



Fig 22: Reddish black patches on the leaf of Ritha plant.

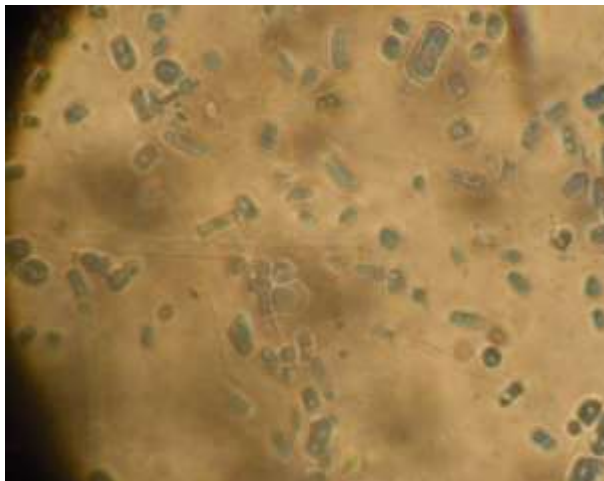


Plate 17: Microscopic view of *Geotrichum candidum*

Xanthomonas campestris (Pammel) Dowson (Xanthomanadales: Xanthomonaceae)

Sample number: Jum1.P3-S2

Isolated number: Ns-Jum1-3-Le

The disease specimen was collected from the leaf of the plant (Fig. 23) from Jum1.P3. The reddish black necrotic spot at the leaf were observed. The further field information of *X. campestris* in Gentian plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The colony is moist, opaque, light yellow in color with smooth margin.

Morphological characteristics

The bacterium is Gram Negative which vegetative cell is straight rod arrange singly with rounded ends (Plate 18).



Fig 23 Reddish brown spots on the leaf of the Gentian Plant



Plate 18: Microscopic view of *X. campestris*

***Pseudomonas syringae* van Hall (Pseudomonadales: Pseudomonadaceae)**

Sample number: Jum1.P2.S2

Isolate number: Ns-Jum1-4-Le

The disease specimen was collected from the leaf of the plant (Fig. 24) from Jum1.P2. The reddish brown necrotic spot at the leaf were observed. The further field information of *P. syringae* in Gentian plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The colony is opaque and slimy.

Morphological characteristics

It shows Gram Negative in Gram stain. The vegetative cell is straight and rod shape(Plate 19)



Fig 24. Reddish brown necrotic spot on the leaf of Gentian plant.

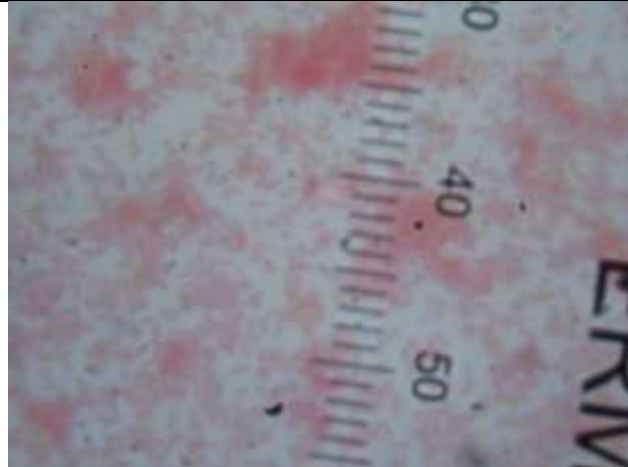


Plate 19: Microscopic view of *P. syringae*

***Alternaria alternata* (Fr.) Keissl. (Pleosporales: Pleosporaceae)**

Sample Number: Jum2.P2.S3

Sample number: Jum2.P1-S2

Isolate Number: Ns-Jum2-5-Le

Isolate number: Ns-Jum2-6-Le

The disease specimen was collected from the leaf of the plant (Fig. 25) from Jum2.P2 and Jum2.P1. The brown irregular leaf spot was observed. The further field information of *A. alternata* in Gentian plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.2 cm. Colony appear circular in form, entire margin with raised elevation, powdery to felty. The colony appear grey to olivaceous green with white margin in obverse and appear dark brown black with light brown periphery.

Morphological characteristics:

The mycelium is sparse, greyish brown, fluffy septate hyphae. The conidia variable in shape often ellipsoidal, obclavate or ovoid, pale to dark brown, 3-7 transverse septa, 1-several longitudinal septa. The size of conidium 28-58x10-25 μm . Conidium is polymorphous formed in branching chains (Plate 20).



Fig 25. Brown irregular spot on leaf of Gentian plant.

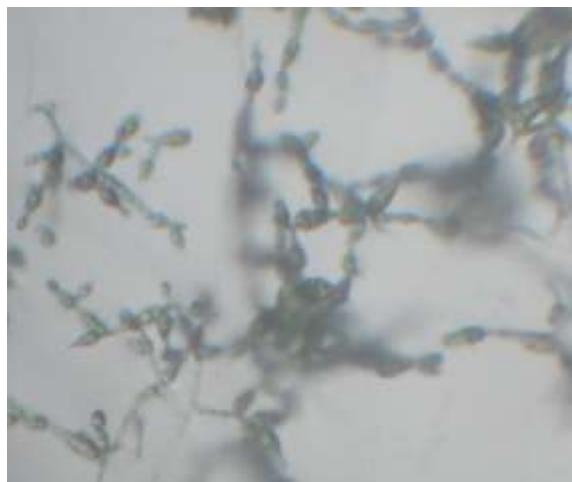


Plate 20. Microscopic view of *Alternaria alternata*

***Fusarium moniliforme* Sheldon (Hypocreales: Nectriaceae)**

Sample no.: Jum1.P3-S1 Sample no.: Jum1.P2-S2
 Isolate no.: Ns-Jum1-7-Ro Isolate no.: Ns-Jum1-8-Le

The disease specimen was collected from the leaf of the plant (Fig. 26) from Jum1.P3. and Jum1.P2. Fungal growth on root and very small whitish leaf spot was observed. The further field information of *F. moniliforme* in Gentian plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristic:

The growth rate of fungus is 4.5 cm. The colony of fungus appears floccose which is circular in form. At obverse view of fungus is white while at reverse view of fungus is peach centre with white periphery.

Morphological characteristics:

The fungus produces hyaline, septate, sparsely branched mycelium. The micro conidia are hyaline, clavate bearing in long chain which size ranges 6-10x2-2.5 µm. The macro conidia are fusoid with 3-6 septa which size ranges 25-35x2.5-4 µm (Plate 21).



Fig 26 Small whitish spot on leaf of Gentian plant



Plate 21 Microscopic view of *Fusarium moniliforme*

***Fusarium dimerium* Penzig (Hypocreales: Nectriaceae)**

Sample number: Jum2.P1-S1

Isolated number: Ns-Jum2-9-St

The diseased specimen was collected from Depalgaun during second field of Jumla district. The stem rot was observed. The further field information of *F. dimerium* in Gentian plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.5 cm. The colony of fungus appears floccose which is circular in form. At obverse view of fungus is white while at reverse view of fungus is peach centre with white periphery.

Morphological characteristics:

The fungus produces hyaline, septate, sparsely branched mycelium. The micro conidia are hyaline, clavate bearing in long chain which size ranges 6-10x2-2.5 μm . The macro conidia are fusoid with 3-6 septa which size ranges 25-35x2.5-4 μm (Plate 22).



Fig 27: Stem rot of the Gentian Plant



Plate 22: Microscopic view of *F.dimerum*

***Curvularia eragrostidis* (Henn) J.A.Mey (Pleosporales: Pleosporaceae)**

Sample number: Jum2.P1-S1

Isolated number: Ns-Jum2-10-St

The diseased specimen was collected from Jum2.P1. The stem rot was observed. The further field information of *C. eragrostidis* in Gentian plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of the fungus colony is 4.1 cm. The colony appears circular in form, compact with flat elevation and entire margin. The colony at obverse appears dark brown with light periphery and dark brown at reverse.

Morphological characteristics:

The hyphae of the fungus are septate and brown whereas conidia are smooth, barrel shape, 3 septa where middle septum is truly median. The conidia of fungus size ranges 17-25 x 9-15 μm (Plate 23).



Fig 28: Stem rot of the Gentian plant



Plate 23: Microscopic view of *Curvularia eragrostidis*

***Alternaria japonica* Yoshii (Order: Pleosporales: Pleosporaceae)**

Sample number: Jum2.P2-S2

Isolate number: Ns-Jum2-11-Le

The diseased specimen was collected from Jum2.P2. The concentric rings on leaf, tan spot with brownish halo later turning into shot holes were observed. The further field information of *A. japonica* in Gentian plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth of fungus is 4.2 cm. The colony appears circular in form. At obverse view the fungus appears olive green while it appears faint olive green at reverse.

Morphological characteristics:

The mycelium is grey, septate and fluffy with golden brown to black conidia. Conidia are usually borne singly or rarely in chains of 2-3, obclavate or ellipsoidal, usually short beak with 2-7 transverse septa and a number of longitudinal and oblique septa. The size of conidia ranges 50-130x14-30 μm (Plate 24).



Fig. 29: tan spot with halo on leaf of Gentian plant

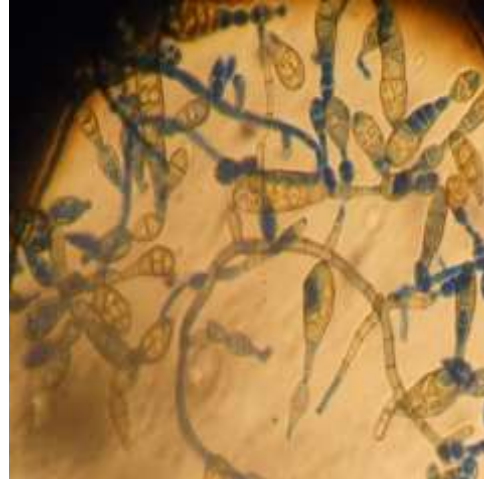


Plate 24. Microscopic view of *Alternaria japonica*

***Pseudomonas aeruginosa* (Schroter) Miquela (Pseudomonadales:Pseudomonadaceae)**

Sample Number: Jum1.P2-S5

Isolate number: Ns-Jum1- 12-So

The diseased specimen was collected from Jum1.P2. The sample is collected from rhizosphere of affected plant. The further field information of *P. aeruginosa* in Gentian plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The colony of bacterium is large and smooth.

Morphological characteristics:

It shows Gram Negative in Gram stain. The vegetative cell is long and rod shape (Plate 25).



Plate 25: Microscopic view of *Pseudomonas aeruginosa*

Erysiphe sp. (Erysiphales: Erysiphaceae)

Sample number: Jum2.P2-S5

The diseased specimen was collected from the leaf of Patmara-2, Raksheydunga during second field of Jumla district. The powdery growth on leaf were observed with superficial moldy growth only on dorsal leaf surface, gray at centre and white at periphery starting from scatter point, later coalescing to form large irregular patches. The further field information of *Erysiphe* sp. in Gentian plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Morphological characteristics:

The hyphae is hyaline and septate bearing erect conidiophores

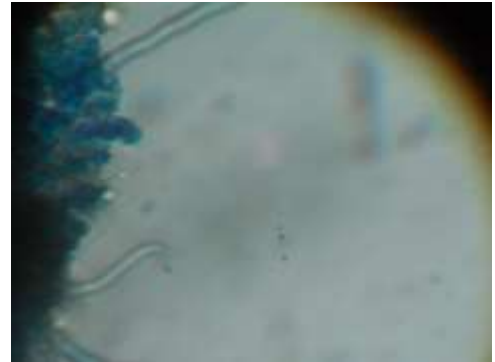


Fig.30: Powdery growth on the leaf of the Gentian Plant Plate26: Microscopic view of *Erysiphe* sp.

3.2.2 Entomological Findings

Blister beetle, *Mylabris cinchorii* Linnaeus (Coleoptera: Meloidae)

General introduction of insects:

The blister beetle, *Mylabris cinchorii* L. was collected from Patmara area during the first visit (Jum1.P3). This insect belongs to Meloidae family of Coleoptera order and is a highly polyphagous insect that feeds on the flowers of several plants. This insect has been reported from many parts of South Asia including Nepal. The further field information of *M. cinchorii* in Gentian plant is described in pest data sheet (Annex III)

Visible damage symptoms in commodity pathway:

Adult beetle is the damaging stage of insects. This insect was observed at the floral parts of gentian plant (Figure 31). The adult generally feeds upon buds and flowers of the plant. So it may have significant impact on the yield.

Field identification of insects:

The blister beetle is a bright colored medium sized insect about 1.5 to 1.8 cm in length. It has characteristic longitudinally three reddish orange and black alternating irregular bands on the forewing (elytra). The insect is highly mobile and was observed feeding on the floral parts of the plant. The eggs are usually laid in the soil and the grub feeds upon other soil dwelling arthropods.

Collection, preservation and transportation of the specimen:

The blister beetle was collected from the floral parts of the Gentian plant using sweep net. The insect was kept at the killing jar for sometime and later transferred to 70% alcohol solution in 50 ml plastic bottle. Later this insect was placed at 30 ml screw capped vial filled with 70% alcohol and transported to the DPR laboratory.

At the laboratory, the specimen (Plate 27) was taken out and the alcohol content was dried using blotting papers. Then the beetle was pinned at the left elytra by standard insect pin and kept for three days for completely drying. After, the dried and pinned specimen was kept at the standard insect box at DPR laboratory.



Fig 31. Blister beetle feeding on Gentian plant

Plate 27: Blister beetle

Green stink bug, *Nezara viridula* (Linnaeus) (Hemiptera: Heteroptera: Pentatomidae)

General introduction of insects:

The green stink bug, *Nezara viridula* (L.) was collected from Depalgaon area during first field visit (Jum1.P1.SP1). This bug is cosmopolitan in warm and temperate regions; highly polyphagous

feeder, attacking many economical food and other crops. The further field information of *N. viridula* in gentian plant is described in pest data sheet (Annex XII).

Visible damage symptoms in commodity pathway:

The adult and nymph are the damaging stages, where the nymphs are observed feeding in groups. The bug damages the plant by sucking the plant sap. They feed upon all plant parts but mostly growing shoots and developing fruit are preferred. The damaged shoots usually wither and in extreme cases they may die. The plant exhibits retarded growth eventually affecting in its quality. This green stink bug was observed at the plant canopy (Fig. 32) and its damage intensity at the field was low.

Field identification of insects:

The green stink bug is a medium sized true bug with prominent shield shaped body (12 to 13 mm). It has uniformly apple green body color with a row of three small white spots between prothorax and sculletum. The eyes are dark red, five segmented antenna with green and red bands. Its presence can be known from the stinking smell exhibiting from its body. The nymphs have varying shape and body color during its development.

Collection, transportation and preservation of the specimen:

The adult green stink bug was collected from the field using sweep net from the plant canopy. The insect was kept at the killing jar for some time and later transferred to 70% alcohol solution in 50 ml plastic bottle. Later this insect was placed at 30 ml screw capped vial filled with 70% alcohol and transported to the DPR laboratory. At the laboratory, the specimen (Plate 12) was taken out and the alcohol content was dried using blotting papers. Then the stink bug was pinned at the left sculletum by standard insect pin and kept for three days for completely drying. After, the dried and pinned specimen was kept at the standard insect box at DPR laboratory.



Fig 32. Green stink bug feeding on Gentian plant Plate 28: Green stink bug

3.2.3 Weed Findings

Common alpine weeds are found associated. They are as follows:

1. *Galinsoga parviflora* L.
2. *Artemisia* sp
3. *Fragaria indica* Andr.
4. *Geranium nepalense* Sweet
5. *Chlorophytum* spp?
6. *Plantago major* L.
7. *Aconitum* sp/
8. *Datura metal* L.
9. *Rumex nepalensis* L.
10. *Bidens* sp?

Table 6: Pest list of cultivated Gentian, *Neopicrorhiza scrophulariflora* (Pannel), D.Y.Hong 2014/15

SN	Pest category	Scientific Name	Common name of Pest	Plant part affected	location
1	Fungi	<i>Alternaria alternata</i> (Fr.) Keissl.	Leaf spot	Leaf	Raksheydunga, Patmara, Jua
2	Fungi	<i>Alternaria japonica</i> (Yoshii)	Leaf spot	Leaf	Raksheydunga, Patmara, Jumla
3	Fungi	<i>Erysiphe sp.</i>	Powdery mildew	Leaf	Raksheydunga, Patmara, Jumla
4	Fungi	<i>Culvularia eragrostidis</i> (Henn) J.A.Mey		Stem	Depalgaun, Jumla
5	Bacteria	<i>Xanthomonas campestris</i> (Pammel) Dowson	Leaf Spot	Leaf	Patmara, Jumla
6	Fungi	<i>Fusarium moniliforme</i> Sheldon	Wilting of root	Leaf, Root	Patmara, Depalgaun, Jumla
7	Fungi	<i>Fusarium dimerum</i> Penzig	Stem rot	Stem	Depalgaun, Jumla
8	Fungi	<i>Geotrichum candidum</i> Link		Leaf	Raksheydunga, Patamara-2, Jumla
9	Bacteria	<i>Pseudomonas syringae</i> van Hall	Wilt	Leaf	Raksheydunga, Jumla
10	Bacteria	<i>Pseudomonas aeruginosa</i> (Schroter) Migula		Soil	Raksheydunga, Jumla
11	Insect	<i>Mylabris cinchorii</i> Linnaeus	Blister beetle		
12	Insect	<i>Nezara viridula</i> (Linnaeus)	Green stink bug		

3.3 Findings of pest survey in Soapnut, *Sapindus mukorossi* (Sapindaceae)

Laboratory Findings:

The pest detection survey on *Sapindus mukorossi* (Ritha) was conducted at Darchula district for three times during vegetative stage, flowering stage and harvesting stage and at the storage. During the first field visit, site selection work was done discussing with concerned stakeholders (DFO, DPRO, local traders, farmers). For this purpose, the procedure mentioned at “survey surveillance guidelines” published by NPPO was followed. With all these background and discussions, the

following fields were identified for the detection survey. The surveyed sites along with stages crop their code number, respective crop stages, GPS are given at Annex XII

First Field:

- Dar1.P1.SP1 : Patan, Baitadi district
- Dar1.P1.SP2 : Patan, Baitadi district
- Dar1.P2.SP1 : Gokuleshwor, Darchula district
- Dar1.P2.SP2 : Nursery of Gokuleshor, Darchula district
- Dar1.P3.SP1 : Khalanga, Darchula district
- Dar1.P4. : Pokhara, Dadeldhura district

Second Field:

- Dar2.P1 : Tallakuna, Gokuleshwor, Darchula district
- Dar2.P2 : Dethala-8, Luitha, Darchula district
- Dar2.P3 : Gwane-1, Pane Baj, Darchula district
- Dar2.P4.SP1 : Nursery, Patan, Baitadi district
- Dar2.P5-SP2 : Patan-4, Lorkha, Baitadi district
- Dar2.P6-S1 : Amarghadi Municipality-7, Pokhara, Dadeldhura district
- Dar2.P7 : Jimkot-4, Baitadi district.

Third Field:

- Dar3. P1 : Pokhara-1, Dadheldura district
- Dar3.P2 : Pokhara-2, Dadheldura district
- Dar3.P3 : Pokhara-7, Amargadhi, Dadheldura district
- Dar3.P4 : Patan, Baitadi district
- Dar3.P5 : Lorkha, Baitadi district
- Dar3.P6 : Runeth, Baitadi district
- Dar3. P7 : luitha,dethala, Darchula district
- Dar3.P8 : Dethala-5, Darchula district
- Dar3.P9.SP3 : Belayeti-4, Gokuleshwor, Darchula district
- Dar3.P10. : Jimkot-4, Baitadai district.

3.3.1 Pathological Findings

Geotrichum candidum Link (Saccharomycetales: Dipodascaceae)

Sample number: Dar3.P9.SP3-S3

Isolate number: Sm-Dar3-1-Fr

The disease specimen was collected from the fruit of the plant (Dar3.P9.SP3-S3) (Fig. 33). The brown spots on the fruit were observed. The further field information of *G. candidum* in Soap nut plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4 cm. The colony appears circular with entire margin, flat elevation with characteristic smell. The colony is white at obverse and creamy white at reverse

Morphological characteristics:

The fungus mycelium is hyaline, septate and branched. The conidia are hyaline, single celled, short & cylindrical with truncate ends (Plate 29).



Fig 33: brown spots on fruit of Soap nut plant

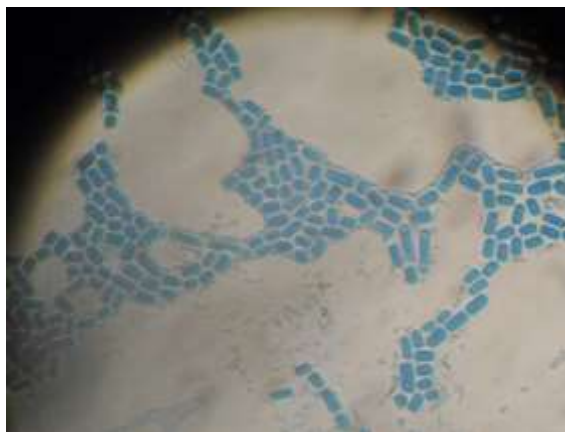


Plate 29: Microscopic view of *Geotrichum candidum*

***Fusarium decemcellulare* Link. (Hypocreales: Nectriaceae)**

Sample number: Dar3. P7-S1

Isolate number: Sm-Dar3-2-Fr

The disease specimens were collected from the fruit of the plant (Fig. 34) from Dethala of Darchula district during third field. The black spots symptoms with wrinkles on fruit were observed in the sample. The further field information of *F. decemcellulare* in Soap nut plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate is 3.2 cm. The colony is irregular, raised and floccose. It appears creamy white in obverse view and yellowish brown with white periphery in reverse view.

Morphological characteristics:

Its micro conidia is oval, aseptate, borne on chain, 10-12x3-4 μ m. Macro conidia is slightly curved, straight to broadly fusoid, 7-10 septate, 50-100 x 5-8 μ m (Plate 30).



Fig 34: black spot and wrinkles on the fruit of Soap nut



Plate 30: *Fusarium decemcellulare*

***Fusarium dimerum* Penzig (Hypocreales: Nectriaceae)**

Sample number: Dar3.P9-S1

Isolate number: Sm-Dar3-3-Fr

The disease specimen was collected from the fruit of Soap nut plant (Fig. 35) of Pokhara-7 of Dadheldura district during the third field visit. The small brown spots were observed on the sample. The further field information of *F. dimerum* in Soap nut plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of the fungus is 2.7 cm. The colony is Floccose, circular in form, umbonate in elevation with entire margin. The fungus on obverse view appear white while in reverse appear light orange.

Morphological characteristics:

The fungus produces hyaline and septate mycelium. Its macro conidia is fusiform that is strongly curved with pointed apex. The fungus consists of 1-3 septa. Its size varies 10-20x3-4 μm . Micro conidia is absent (Plate 31).



Fig no. 35: small brown spot on fruit



Plate 31: Microscopic view of *F.dimerum*

Bipolaris sorokiniana (Sacc) Shoem.(Pleosporales: Pleosporaceae)

Sample number: Dar1.P3.SP1-S8

Isolated number: Sm-Dar1-4-St

The disease specimens were collected from the stem of the plant from Khalanga area of Darchula district during first field visit. Rust like spots on stem was observed. The further field information of *B. sorokiniana* in Soap nut plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth of the fungus is 4.5 cm. Its colony is irregular and flat. The obverse view of fungus is grayish black while reverse view is grayish black with dark brown ridges

Morphological characteristics

The fungus produces septate and hyaline mycelium which bears unbranched conidiophores. The conidia are ellipsoid, dark brown, smooth walled with ends rounded which is broad at middle. It bears 3-10 distosepta, which size ranges 40-100x17- 23 µm (Plate 32)..



Plate 32: Microscopic view of *Bipolaris sorokiniana*

Fusarium oxysporum Schlecht. (Hypocreales: Nectriaceae)

Sample number: Dar1.P3.SP1-S2

Isolate number: Sm-Dar1-5-St

Sample number: Dar2.P2.SP-2

Isolate number: Sm-Dar2-8-Fr

Sample number: Dar3.P8-S1

Isolate number: Sm-Dar3-11-Fr

Sample number: Dar2.P1 -S4

Isolates number: Sm-Dar2-6-Fr

Sample number: Dar2.P6. S1

Isolate number: Sm-Dar2-9-Fr

Sample number: Dar3.P4-S1

Isolate number: Sm-Dar3-7-Fr

Sample number: Dar3.P6-S1

Isolate number: Sm-Dar3-10-Fr

The disease specimens were collected from the stem of the plant (Fig. 36) from Lorkha, Patan and Runeth of Baitadi district during first field visit and from PaneBaj, Dethala, and Pokhara, Karkarley of Darchula district during second field visit and from Runeth and Lorkha of Baitadi district during third field visit. The leaf curling, discoloration and white, brown spots on fruit were

observed (Fig. 37). The further field information of *F. oxysporum* in Soap nut plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.5 cm. Its colony is circular, floccose, crateriform in elevation with entire margin. The obverse and reverse view of fungus is pinkish white to purple.

Morphological characteristics:

The fungus produces aerial, septate and branched mycelium. The fungus micro conidia is oval to ellipsoidal which size ranges 5-12x2.5-3.5 μm while macro conidia is fusiform that is slightly curved with 3-5 septa that size ranges 24-45x3-5 μm (Plate 33).



Fig 36. Brownish spots and discoloration of fruit

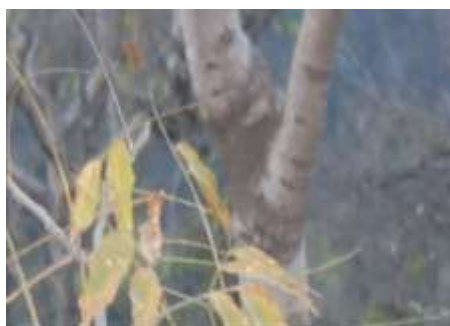


Fig 37. Leaf curling and yellowing



Plate 33: Microscopic view of *Fusarium oxysporum*

Alternaria radicina Meier, Drechsler & E.D. Eddy (Pleosporales:Pleosporaceae)

Sample number: Dar2.P3. S6 Sample number: Dar2.P5-SP-2 Sample number: Dar2.P1-S4
Isolated number: Sm-Dar2-12-Fr Isolate number: Sm-Dar2-13-Le Isolate number: Sm-Dar2-14-Fr

The disease specimens were collected from the fruit of the plant (Fig. 38) from Darchula and Baitadi districts during second field visit and also from leaf of the Soap nut plant during second field visit in Patan of Baitadi district. Small powdery black pustules on leaf and brown, dark spots

were observed on the fruits (Fig. 39). The further field information of *A. radicinain* in Soap nut plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth of fungus is 4.3 cm. The colony appears circular in form, flat elevation with entire margin. The colony of fungus appears brownish green at obverse and dark brown with light periphery at reverse.

Morphological characteristics:

The fungus produces greyish, fluffy mycelium in which black shiny conidia appears single or in pairs arranged in short chains. The conidia are variable in shape which is often ellipsoidal or may be obclavate or ovoid. Color of conidia is pale to dark brown with 3-7 transverse septa and one or several longitudinal or oblique septa which size is 27-57x9-27 μm (Plate 34).



Fig 38: Dark spot on leaf of Soap nut plant



Fig 39: Blackish brown spot on fruit of Soap nut

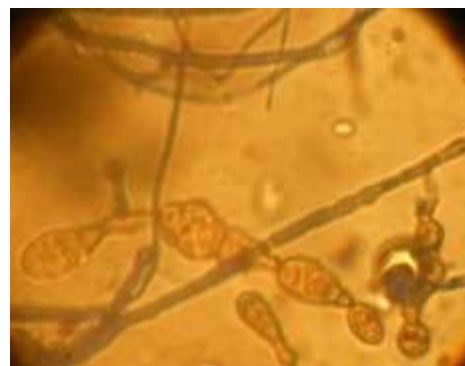


Plate 34: Microscopic view of *Alternaria radicina*

***Alternaria alternata* (Fr.) Keissl. (Pleosporales: Pleosporaceae)**

Sample number: Dar2-P6-S1 Sample number: Dar3. P1-S1 Sample number: Dar3.P3-S1
Isolate number: Sm-Dar2-15-le isolate number: Sm-Dar3-16-Le Isolate number: Sm-Dar3-17-Fr
Sample number: Dar3.P6-S1 Sample number: Dar3.P5-S1 Sample number: Dar3.P8-S1
Isolate number: Sm-Dar3-18-Fr Isolate number: Sm-Dar3-19-Fr Isolate number: Sm-Dar3-20-Fr
Sample number: Dar3.P9.SP3-S3
Isolated number: Sm-Dar3-21-Fr

The disease specimen was collected from the leaf of the Soap nut plant (Fig. 40 and 41) from Pokhara of Dadeldura during second field visit and from the fruits of Pokhara-1 and Pokhara-2 of Dadheldura district, Patan and Lorkha of Baitadi district, Dethala-5 and Belayeti-4, Gokuleshwor

of Darchula District during second and third field visit. The brown powdery spots on leaves, grey, brown spots on fruit along with discoloration of fruit were types of symptoms observed. The further field information of *A. alternata* in Soap nut plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.2 cm. Colony appear circular in form, entire margin with raised elevation, powdery to felty. The colony appear grey to olive green with white margin in obverse and appear dark brown black with light brown periphery.

Morphological characteristics::

The mycelium is sparse, grayish brown, fluffy and hyphae septate. The conidia variable in shape often ellipsoidal, obclavate or ovoid, pale to dark brown, 3-7 transverse septa, 1-several longitudinal septa. The size of conidia ranges from 28-58x10-25 µm. Conidia are polymorphous formed in branching chains (Plate 35).



Fig 40: *A. alternata* leaf spots at the Soap nut Plant



Plate 35: Spores of *A. alternata* of Soap nut plant



Fig 41 brown -black spot on fruit of the Soap nut plant

***Alternaria longissima* Deighton & MacGarvie. (Pleosporales: Pleosporaceae)**

Sample number: Dar2.P4.SP-1

Isolate number: Sm-Dar2-22-le

The disease specimen was collected from the leaf of the Soap nut plant (Fig. 42) from nursery of Patan of Baitadi district during second field visit. The discoloration of fruit with brown and black spots was observed on the fruit. The further field information of *A. longissima* in Soap nut plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth of fungus is 4.7 cm. The shape of colony appears circular in form, entire in margin with raised elevation. The fungus colony a olivaceous to dark grey in obverse view and dark grey in reverse view (Plate 36).

Morphological characteristics:

The fungus consist of grey fluffy mycelium which is partly superficial and partly immersed where conidiophores septate, sparingly branched with smooth wall that wide range to 2.5-3 μm . Conidia solitary or in short monopodial chains; elongate cylindrical or somewhat irregular in outline, beakless or with a long beak, thin-walled, pale brown to almost hyaline, 30-120x10-21 μm . The conidium body with many transverse septa, usually 5-10, constricted at most of the transverse septa, smooth-walled; conidium beak relatively thick, with many septa, unbranched, tapering into the conidium (Plate 36).



Fig 42: *A. longissima* on the leaf of Soap nut Plant



Plate 36: Microscopic view of *A. longissima*

Cladosporium sp. (Capnodiales: Davidiellaceae)

Sample Number: Dar3. P1-S1

Isolate number: Sm-Dar3-23-Fr

The disease specimens were collected from the fruit of the plant (Fig. 43) from Pokhara-1 of Dadheldura district during third field visit. The discoloration of fruit along with brown spot was observed. The further field information of *Cladosporium* sp.in Soap nut plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 2.1 cm. The colony consists irregular in form, compact and with raised elevation. The colony of fungus at obverse appears army green and black at reverse (Plate 37).

Morphological characteristics:

The fungus produces aerial mycelium with coiled hyphae. The conidia appear in 3-5 chain, shape ranges from ovate to cylindrical with olive brown color. The size of conidia ranges 3-4 μm (Plate 37).



Fig 43: Greenish -brown spots on the fruit of Soap nut plant



Plate 37: Spores of *Cladosporium sp*

Colletotrichum lindemuthianum Sacc & Magnus, Michelia (Sordariomycetidae: Glomerellaceae)

Sample number: Dar2.P2.SP-2

Isolate number: Sm-Dar2-24-Fr

The disease specimens were collected from the fruit of the plant (Fig. 44) from Darchula district during second field visit. The discoloration of fruit with grayish white spot was observed. The further field information of *C. lindemuthianum* in Soap nut plant is described in pest data sheet(Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics

The colony is irregular, cottony and floccose. The colony at obverse appears gray centre with white periphery and while in reverse appears greyish black centre with white periphery.

Morphological characteristics

The mycelium is hyaline and consist septa. The colony is hyaline, oblong to dumble shaped, 1-celled with ends rounded which measures 9-14x3-4 μm (Plate 38).



Fig 44: Grayish patches on the fruit of Soap nut Plant

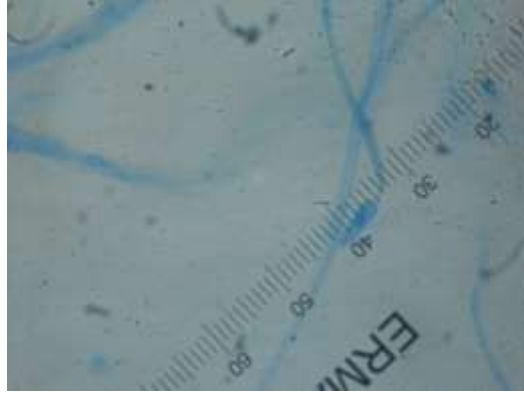


Plate 38: Microscopic view of *Colletotrichum lindemuthianum*

***Pseudomonas syringae* (van Hall 1904) (Pseudomonadales: Pseudomonaceae)**

Sample number: -S1

Sample number: -S1

Isolate number: Sm-Dar3-25-Fr

Isolate number: Sm-Dar3-26-Fr

The disease specimens were collected from the fruit of the plant (Fig. 45) from Dar3.P4 and Dar3.P1. The discoloration of fruit with grayish white spot was observed.

Cultural characteristics:

The colony is opaque and slimy.

Morphological characteristics:

It shows Gram Negative in Gram stain. The vegetative cell is straight and rod shape (Plate 39).



Fig 45: Grayish spot in the Soap nut fruit.

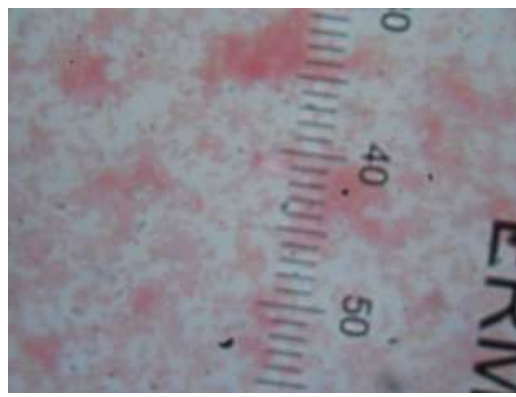


Plate 39: Microscopic view of *Pseudomonas syringae*

3.3.2 Entomological Data (Identification and Interpretation)

Lygaeid bug, *Lygaeus civilis* Wolff (Hemiptera: Heteroptera: Lygaeidae)

General introduction of insects:

The lygaeid bug, *Lygaeus civilis* Wolff was collected from Dar1.P1.SP2 and Dar2.P4.SP1 from the nursery of Soap nut plants. This bug belongs to Lygaeidae family of Hemiptera order and is a polyphagous insect which damages the seeds and plant sap. Though it is an insect of minor importance but in case of heavy attack, it may affect the seedlings at nursery. The further field information of *L. civilis* in soap nut plant is described in pest data sheet (Annex XIII).

Visible damage symptoms in commodity pathway:

Generally the adult lygaeid bug is the nectar feeder and the nymphs suck the plant sap from young plants. This insect was observed at the nursery of forest office during first and second visit. The nursery consists of different forest plants including Soap nut and the nymphs were observed feeding on young plants of soap nut also. They feed on groups at the tender parts of nursery plants (Figure 46). Similar observation was also observed on the seeds of Soap nut tree (Figure 47), but its economic damage was not notable. According to the local personnel, the insect feeds more during morning and evening time at the nursery plants rather than day time.

Field identification of insects:

This is a bright colored medium sized bug about 15 to 20 mm in length. They have a characteristic red-black pattern, fully developed wings and long, powerful legs. The forewing forms an 'X' shape but does not quite meet in the middle. Nymphs have bright red colored abdomens and look like the adults except they do not have full wings and differ in color pattern. The nymphs could be seen near the plant and are very active.

Collection, preservation and transportation of the specimen:

The adult and nymphs of lygaeid bugs are hard to collect because of its active behaviour. The adults were collected using the sweep net while the nymphs were collected by swift catch method using 250 ml plastic bottle. The insects were kept at the killing jar for some time and later transferred to 70% alcohol solution in 50 ml plastic bottle. Later this insect was placed at 30 ml screw capped vial filled with 70% alcohol and transported to the DPR laboratory.

At the laboratory, the specimen (Plate 40) was taken out and the alcohol content was dried using blotting papers. The adult bugs were pinned at the left scutellum by standard insect pin and kept

fom three days for completely drying. After, the dried and pinned specimen was kept at the standard insect box at DPR laboratory. The nymphs were placed in 20 ml screw capped glass vials with 70% alcohol.



Fig 46: Lygaeid bug feeding on soap nut nursery plants in group



Fig 47: Lygaeid bug feeding on soap nut plants at nursery



Fig 48: Lygaeid bug feeding on soap nut fruits



Plate 40: Lygaeid bug

Stem borer (Unidentified)

General introduction of insects:

The incidence of this damage was observed at Jimkot, Baitadi area during second (Dar2.P7) and third visit (Dar3.P10) on soap nut trees. The field observation of this symptom in soap nut plant is described in pest data sheet (Annex XIII).

During the field observation, some wilted and dead leaves were noticed in one of the soap nut tree. The damage was observed at the auxillary branch and the age of the tree was nearly eight to nine years. This damage incidence was closely watched, where few exit holes (Fig. 49) was located at

the branch. The stem was cut with the grafting knife, where the symptom of hollow branches with remains of excreta was observed (Fig. 50). The diameter of the stem was 2.4 cm, while the bored hole was about 14 cm long with 6 mm diameter.

As per the damage symptom, the damaging insect might be coleopteran borer. This evidence was also given by the nearby people as they have noticed some beetle during early spring season. According the farmers, this type of damage is noticed in few soap nut trees without much economical damage.

The damaged branch of the soap nut tree was cut down and the damaged part was separated from the branch. The damaged twig was wrapped with cotton roll and kept at large sized plastic bottle. This specimen is preserved at DPR laboratory.

Stem borer (Unidentified)

The incidence of this damage was observed at almost all sites during second and third visit on fruits of soap nut at standing trees and storage. The field observation of this symptom in soap nut plant is described in pest data sheet (Annex XIII).

The borer damage symptoms were found at fruit of Soap nut (Fig. 51). This damage symptom was observed both at standing trees and at the local collectors' storage places. A small hole (about 5 mm diameter) was observed at the outer surface of the Soap nut fruit (Fig. 52). The hole was observed even at the seed of the fruit. During our observation, only one hole was found in one soap nut fruit. The damaged fruit were collected and placed at paper envelope. These specimens are preserved at DPR laboratory.



Fig 49: Exit hole of borer on soap nut tree



Fig 50: Stem borer damage at soap nut tree



Fig 51: Borer damage symptoms on soap nut fruit



Fig 52: Borer hole on soap nut fruit

3.3.3 Weed Data

Associated species of *Ritha Sapindus mukorossi* (Salyan District)

1 Toona ciliata M. Roem.

Toona ciliata is a forest tree in the mahogany family. It is commonly known as the **red cedar**, **toon** or **toona**, **Indian cedar**, **Australian red cedar** or the **Queensland red cedar**. It is also sometimes known as **Indian mahogany**.

It grows best in an environment with high light levels, however in the relative darkness of the rainforest understory, it is less susceptible to attack by the Cedar Tip Moth, which lays its eggs on the tree's leading shoot, allowing the larvae to burrow into the stem, causing dieback and a multi-branched tree with little commercial value.^[10] The red cedar is widely planted in subtropical and tropical parts of the world as a shade tree and for its fast-growing aspect.

Toona ciliata reproduces by seed. It is a prolific seed producer and establishes readily.

2. Quercus leucotrichophora is a tree belonging to Family Fagaceae; commonly known as **Banjh oak**. In Nepal, it is known as *Banjhi*, *Rainj*, *Khasarant*, *Tikhe bhanjh* in Standard Nepali and *Sulsing* in Tamang language. Some authors named it as *Quercus incana* Roxburgh.

Table 7: Pest list of cultivate Soap nut, , *Sapindus mukorossi*, Gaetr. 2014/15

S. N.	Pest Category	Scientific Name of Pest	Common Name of Pest	Plant Parts Affected	Location
1	Fungi	<i>Alternaria alternata</i> (Fr.) Keissl.	leaf spot	Leaf, Fruit	Amargadhi, Pokhara, Dadeldhura; Dethala, Darchula; Patan, Bitadi; Belayeti, Gokuleshwor, Darchula
2	Fungi	<i>Fusarium oxysporum</i> Schlecht.	Rot	Stem, Fruit	Tallakuma, Gokuleshwor; Dethala-9, Karkaley; Gwane-1, Panebaj; Patan, Lorkha, Dethala-5, Darchula
3	Fungi	<i>Bipolaris sorokiniana</i> (Sacc) Shoem		Stem	Khalanga, Darchula
4	Fungi	<i>Colletotrichum gloeosporioides</i> DickSacc & Magnus, Michelia	Anthracnose	Fruit	Dethala-8, luitha, Darchula
5	Fungi	<i>Geotrichum candidum</i> Link		Fruit	Belayeti-4, Gokuleshwor, Darchula
6	Fungi	<i>Fusarium decemcellulare</i> link.		Fruit	Luitha, Dethala, Darchula
7	Fungi	<i>Fusarium dimerium</i> Penzig		Fruit	Pokhara-7, Dadeldhura
8	Fungi	<i>Alternaria radicina</i> , Meier, Drechsler & E.D. Eddy	Leaf spot	Fruit, Leaf	Gwane, Panebaj; Patan, Lorkha, Baitadi; Tallakuna, Gokuleshwor, Darchula
9	Fungi	<i>Alternaria longissima</i> Deighton & MacGAvie,	Leaf spot	Leaf	Nursery, Patan, Baitadi
10	Fungi	<i>Cladosporium sp.</i>		Fruit	Pokhara, Dadeldhura
11	Bacteria	<i>Pseudomonas syringae</i> (Van Hall)	Wilt	Fruit	Patan, Pokhara, Baitadi
12	Insect	<i>Lygaeus civilis</i> Wolff	Lygaeid bug,		
13	Insect	<i>Lygaeus civilis</i> Wolff	Lygaeid bug	seed	
14	Insect	Stem borer			
15	Insect	Stem borer			

3.4 Findings of pest survey in Chiretta ,*Swertia chirayita*,

The pest detection survey on Chiretta was conducted at Ilam district. During the first field visit, site selection work was done discussing with concerned stakeholders (DFO, DPRO, local traders, farmers). For this purposes, the procedure mentioned in “Technical Guidelines for Detection Survey of Plant Pests in Nepal” published by NPPO was followed. With all these background and discussions, the following fields were identified for the detection survey:

First field:

- Ila 1.P1.SP1 : Maipokhari, Nursery
- Ila 1.P1.SP2 : Maipokhari, wild
- Ila 1.P2 : Solbung, Maipatal,Ilam
- Ila1.P3 : Maimajhuwa, Ilam
- Ila 1.P4.SP1 : Bagkhor, Chamaita, Ilam
- Ila 1.P4. SP2 : Chaitegaun, chamaita, Ilam

Second Field:

- Ila2.P1.SP1 : Solbung-1, Maipatal
- Ila2.P1.SP2 : Solbung-1, Maipatal
- Ila2.P1.SP3 : Solbung-1, Maipatl
- Ila2.P2 : Maimajhuwa-2, Femeguru
- Ila2.P3 : Chamaita-7, Bagkhor

3.4.1 Pathological Findings

***Fusarium solani* (Mart.) Sacc. (Hypocreales:Nectriaceae)**

Sample number: Ila1.P1.SP1-S1 Sample number: Ila1.P4.SP2
Isolate number: Sc-Ila1-1-Ro Isolate number: Sc-Ila1-2-Ro

This diseased plant was collected from Ila1P1 and Ila12P4 during first field visit. The disease specimen was collected from the root of the plant (Fig. 53). The white, cottony fungal growth in the soil of roots was observed with wilting of affected plant. The further field information of *F. solani* in Chiretta plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 3.2 cm. The colony of fungus is regular, floccose with raised elevation. At obverse the fungus is white to cream while reverse view is yellowish with white periphery.

Morphological characteristics:

The mycelium is hyaline, aerial and septate. The micro conidia is cylindrical to oval which size ranges 8-15 X 2-4 µm and macro conidia is inequilaterally fusoid with 1-5 septa which size ranges 35-55x4.6 -6 µm (Plate 41).



Fig 53: white, cottony fungal growth in the soil of roots



Plate 41. : Microscopic view of *F.solani*

Fusarium moniliforme Sheldon.. (Hypocreales : Nectriaceae)

Sample number: Ila1.P3-S1 Sample number: Ila2.P1.SP1-S2

Isolate number: Sc-Ila1-3-Le Isolate number: Sc-Ila2-4-Le

This diseased sample was collected from the leaf of Chiarayita from Ila1P3, Ilam during first and Ila2P1 of Maipatal during second field visit. The brown necrotic lesions on leaf were observed with wilting of affected leaves and xylem browning was observed. The further field information of *F. moniliforme* in Chiretta plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.5 cm. The colony of fungus appears floccose which is circular in form. At obverse view of fungus is white while at reverse view of fungus is peach centre with white periphery (Plate 42).

Morphological characteristics

The fungus produces hyaline, septate, sparsely branched mycelium. The micro conidia are hyaline, clavate bearing in long chain which size ranges 6-10x2-2.5 μm . The macro conidia is fusoid with 3-6 septa which size ranges 25-35x2.5 -4 μm .



Fig 54: Brown necrotic lesions on leaf



Fig 55: Xylem browning



Plate 42: Microscopic view of *F. moniliforme*

***Fusarium oxysporum* Schlecht. (Hypocreales : Nectriaceae)**

Sample number: Ila1.P3-S2

Isolate number: Sc-Ila1-5-Ro

The disease specimen was collected from the root of the plant from Ila1P3 (Fig. 56). The white, cottony fungal growth in the soil of roots was observed with wilting of affected plant.

The further field information of *F. oxysporum* in Chiretta plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.5 cm. Its colony is circular, floccose, crateriform in elevation with entire margin. The obverse and reverse view of fungus is pinkish white to purple (Plate 43).

Morphological Characteristics:

The fungus produces aerial, septate and branched mycelium. The fungus micro conidia is oval to ellipsoidal which size ranges 5-12x2.5-3.5 μm while macro conidia is fusiform that is slightly curved with 3-5 septa that size ranges 24-45x3-5 μm .



Fig 56: The white, cottony fungal growth in the oil of roots

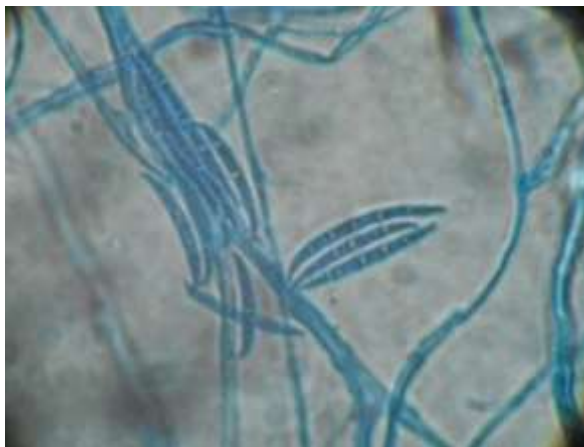


Plate 43: Microscopic view of *F. oxysporum*

Alternaria alternata (Fr.) Keissl. (Pleosporales: Pleosporaceae)

Sample number: Ila1.P4.SP2-S1

Isolate number: Sc-Ila1-6-Le

The disease specimen was collected from the leaf of the plant from Ila1P4 (Fig. 57). The brown spots at the leaf were observed with tip drying of affected leaves. The further field information of *A. alternata* in Chiretta plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.2 cm. Colony appear circular in form, entire margin with raised elevation, powdery to felty. The colony appear grey to olive green with white margin in obverse and appear dark brown black with light brown periphery (Plate 44).

Morphological characteristics:

The mycelium is sparse, grayish brown, fluffy, septate hyphae. The conidia is variable in shape often ellipsoidal, obclavate or ovoid, pale to dark brown, 3-7 transverse septa, 1-several longitudinal septa. The size of conidia ranges from 28-58x10-25 μm . The conidia are polymorphous formed in branching chains.



Fig 57: leaf spots at the Chiretta plant



Plate 44: Spores of *A. alternata* of Chiretta plant

***Bipolaris sorokiniana* (Sacc) Shoem.(Pleosporales: Pleosporaceae)**

Sample number: Ila1.P3-S3 Sample number: Ila1.P3-S4

Isolate number: Sc-Ila1-7-Le Isolate number: Sc-Ila1-8-Le

This disease sample was collected from Ila1P3 during first visit. Diseases specimen was collected from the leaf of plant (Fig. 58). The brown spots at the leaf were observed. The further field information of *B. sorokiniana* in Chiretta plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural morphological characteristics:

The growth of the fungus is 4.5 cm. Its colony is irregular and flat. The obverse view of fungus is grayish black while reverse view is grayish black with dark brown ridges (Plate 45). The fungus produces septate and hyaline mycelium which bears unbranched conidiophores. The conidia is ellipsoid, dark brown, smooth walled with ends rounded which is broad at middle. It bears 3-10 distosepta, which size ranges 40-100x17- 23 μm .



Fig 58: Brown spots at the leaf



Plate 45: Microscopic view of *B. sorokiniana*

Verticillium albo-atrum Reinke & Berthold. (Plectosphaerellaceae)

Sample number: Ila2.P1.SP1.S5

Sample number: Ila2.P3.SP1.S1

Isolate number: Sc-Ila2-9-Le

Isolate number: Sc-Ila2-10-St

This disease sample was collected from Ila2.P1 and Ila2.P3 during second visit. The disease specimen was collected from the leaf and stem of the plant (Fig. 59). The leaf blotch and wilting of stem were observed. Such symptom was found severe in condition. The further field information of *V. albo-atrum* in Chiretta plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural morphological characteristics:

The growth rate of the fungus is 5.9 cm. The growth of fungus is regular, cottony and floccose with raised elevation. The obverse view of the fungus is light yellow to light brown while reverse view is light yellow (Plate 46). The mycelium is hyaline and septate. Its conidia are hyaline, ellipsoidal to sub cylindrical. It arises singly at the apices of verticillate phialides.



Fig 59: leaf blotch and wilting of stem

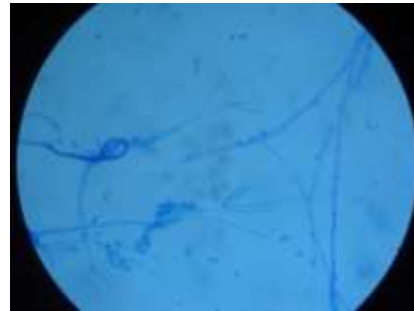


Plate 46: Microscopic view of *V. albo-atrum*

Colletotrichum gloeosporoides Dickman (Glomerellales : Glomerellaceae)

Sample number: Ila2.P1.SP1-S5

Isolate number: Sc-Ila2-11-Le

This disease sample was collected from first plot of Ila2.P1 of Ilam district during second field visit. The disease specimen was collected from the leaf of the plant (Fig. 60) and leaf blotch and spots were observed. The further field information of *C. gloeosporoides* in Chiretta plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 2 cm. The fungus colony is floccose, loose and filamentous. The colony appears dull white at obverse and orange with white periphery at reverse.

Morphological characteristics:

The fungus produces hyaline, white and shiny mycelium. The conidia are non-septate, cylindrical with ends rounded. The size of conidia measures 6-14x3-4 μm (Plate 47).



Fig 60: Leaf blotch and spots

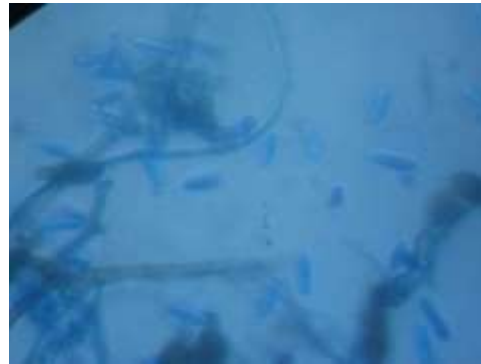


Plate 47: Microscopic view of *C. gleosporoids*

Trichoderma harzianum Rifai (Hypocreales: Hypocrealeace)

Sample number: Ila2.P1.SP1-S6

Isolate number: Sc-Ila2-12-St

This disease sample was collected from Ila2.P1 of Ilam district during second field visit. The disease specimen was collected from the stem of the plant (Fig. 61) and xylem browning and wilting of stem were observed. The further field information of *T. harzianum* in Chiretta plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 3.5 cm which colony is flat, filamentous with entire margin. The colony consists of patches of green conidia. At obverse view, the colony is pale yellow and at reverse view, the colony is creamy white with green patches.

Morphological characteristics:

The mycelium of the fungus is hyaline and septate where conidiophores are highly branched. The conidia are hyaline, ovoid, one celled borne in small terminal cluster(Plate48)



Fig 61: Xylem browning and wilting of stem

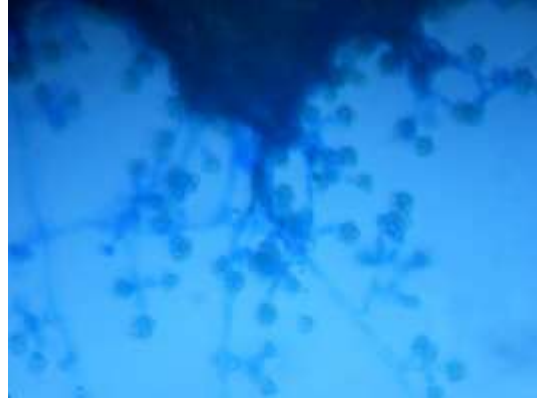


Plate 48: Microscopic view of *T. harzianum*

***Geotrichum candidum* Link.(Schacromycetales: Dipodascaceae)**

Sample number: Ila2.P1.SP3-S1

Isolate number: Sc-Ila2-13-Le

This disease sample was collected from Ila2P1 of Ilam during second visit. The disease specimen was collected from the leaf of the plant (Fig. 62) and leaf spot and blight on leaf were observed. The further field information of *G. candidum* in Chiretta plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics

The growth rate of fungus is 4 cm. The colony appears circular with entire margin, flat elevation with characteristic smell. The colony is white at obverse and creamy white at reverse

Morphological characteristics

The fungus mycelium is hyaline, septate and branched. The conidia is hyaline, single celled, short, cylindrical with truncate ends (Plate 49)..



Fig 62: leaf spot and blight on leaf

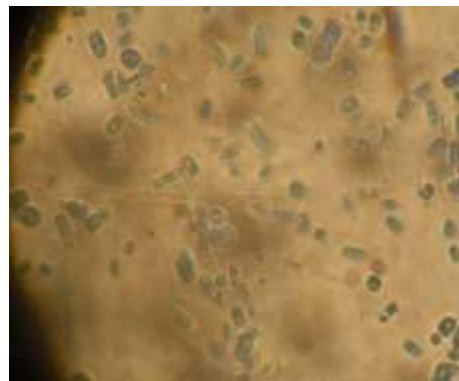


Plate 49: Microscopic view of *G. candidum*

Agrobacterium tumefaciens Smith & Townsend Rhizobiales-Rhizobiaceae)

Sample number: Ila2.P1.SP1.S7

Isolate number: Sc-Ila2-14-St

This disease sample was collected from Ila2.P1 of Ilam during second visit. The disease specimen was collected from the stem of the plant (Fig. 63). The crown gall on stem was observed plant). The further field information of *A. tumefaciens* in Chiretta plant is described in pest data sheet (Annex XIII). The cultural and morphological characteristics are:

Cultural characteristics:

The colony of bacterium is creamy which give characteristic pink to brick red in MacConkey Agar.

Morphological characteristics:

The fungus show Gram Negative in Gram stain which vegetative cell of bacterium is small, rod shape which is arrange singly (Plate 50).



Fig 63: Crown gall on stem of the Chiretta Plant



Plate 50: Microscopic view of *A.tumefaciens*

Verticillium sp. (Hypocreales: Plectosphaerellaceae)

Sample no.: Ila.2.P3.SP1-S2

Isolate no.: Sc-Ila2-15-Le

This disease sample was collected from Ila2.P3 of Ilam district during second field visit. The disease specimen was collected from the leaf of the plant (Fig. 64). The leaf spot with wilting of stem were observed. This symptom was found severe in condition. The further field information of *Verticillium* sp. in Chiretta plant is described in pest data sheet (Annex XIII).

Cultural characteristics:

The growth rate of the fungus is 5.9 cm. The growth of fungus is regular, cottony and floccose with raised elevation. The obverse view of the fungus is light yellow to light brown while reverse view is light yellow

Morphological characteristics:

The mycelium is hyaline and septate. Its conidia are hyaline, ellipsoidal to sub cylindrical. It arises singly at the apices of verticillate phialides (Plate 51).



Fig 64: leaf spot and wilting of stem

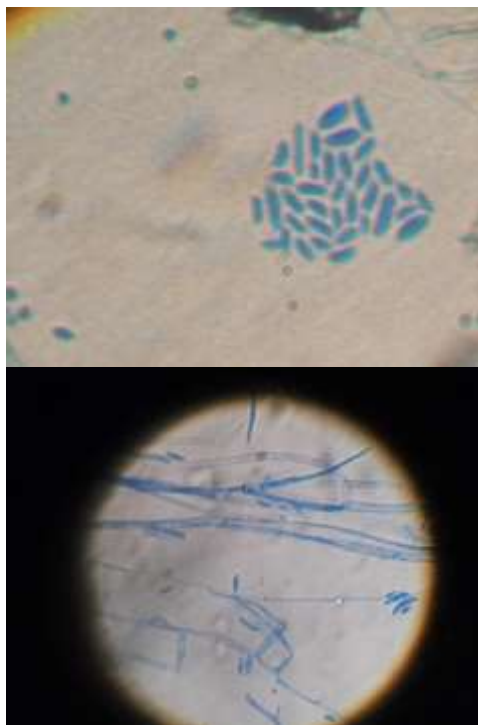


Plate 51: Microscopic view of *Verticillium*.
sp

3.4.2 Entomological Findings

Stem borer – 1 (Unidentified)

The incidence of this damage was observed at almost all the observation sites during second visit at almost all survey sites of Ilam district. The field observation of this symptom in Chiretta plant is described in pest data sheet (Annex XIII).

During the field observation, some wilted/ dead leaves and some dead shoots were noticed. This damage was observed at the main stem of the plant. This damage incidence was closely watched, where few exit holes (Fig. 65) was located near to the node of the stem. The stem was cut with the grafting knife, where the symptom of hollow stem with remains of excreta was observed.

This symptom was observed in almost all fields we visited and the damage intensity is high. Mostly the plants ready to be harvested were affected by the borers. During the observation, we observe two types of boring pattern on the basis of boring length on the stem. The first was small length bores (about 4.5 cm in length) which do not exceed the length of the internodes (Fig. 66). The exit holes are also observed at the upper end of the internodes. This could be the damage by short life cycle borers especially of Lepidoptera order. The damaged branch of the chiretta plant was cut down and the damaged part was separated from the branch. The damaged branch was wrapped with cotton roll and kept at large sized plastic bottle. This specimen is preserved at DPR laboratory.

Stem borer – 2 (Unidentified)

The incidence of this damage was observed at almost all the observation sites during second visit at almost all survey sites of Ilam district. The field observation of this symptom in Chiretta plant is described in pest data sheet (Annex XIII).

During the field observation, some wilted/ dead leaves and some dead shoots were noticed. This damage was observed at the main stem of the plant. This damage incidence was closely watched; where few exit holes were located near to the node of the stem. The stem was cut with the grafting knife, where the symptom of hollow stem with remains of excreta was observed.

This symptom was observed in almost all fields we visited and the damage intensity is high. Mostly the plants ready to be harvested were affected by the borers. This boring pattern in this type of damage was longer length bores (about 15 cm in length) which covers more than one internode (up to three internodes) of the stem (Fig. 67 and 68). This could be the damage by long life cycle borer especially Coleopteran borer. The number of long bore on the stem was more than the smaller one described before. As per the local people, they have observed some weevil like insect during the spring season.

The damaged branch of the Chiretta plant was cut down and the damaged part was separated from the branch. The damaged branch was wrapped with cotton roll and kept at large sized plastic bottle. This specimen is preserved at DPR laboratory.



Fig 65: Exit holes of borer at the stem of chiretta plant



Fig 66: Exit holes of borer at the stem of chiretta plant



Fig 67. Stem borer damage at the stem of chiretta plant



Fig 68. Stem borer damage at the stem of chiretta plant

3.4.3 Weed Data

The most common associate reported species of *Swertia chirayita* are: Bhuin Kaphal (*Fragaria indica*), Bukephool (*Anaphilis triplinervis*), Chari Amilo (*Oxalis corniculata*), Dubo (*Cynodon dactylon*), Ghans (*Digitaria adecendens*), (*Desmodium oxyphyllum*), (*Elsholtzia strobilifera*), Titepati (*Artemesia vulgaris*), *Anemone obtusiloba*, *Bidens* spp, *Eupatorium adenophorum*, *Viola* spp., *Rhododendron arboretum* and *Acer* spp. (Phoboo and Jha, 2010; Bhatt et al., 2006; Sharma et al., 2011). www.nepl.com.np/resources/information_sheets/chiraito.pdf

Present project also found following weeds associated with Chirayita plants.

1. *Cyperus sp*
2. *Persicaria hydropiper(L.) Delabre*
3. *Anaphalis contorta(D. Don) J.D.Hooker*
4. *Gaultheria fragrantissima Wall.*
5. *Gentiana sp*
6. *Fragaria nubicola Lindl. Ex Lacaita*
7. *Zanthoxylum nepalensis ?*
8. *Kyllinga brevifolia Rottb.*
9. *Cynodon dactylon (L.)Pers.*
10. *Ageratina adenophora (Spreng.) King & H.Rob*
11. *Laphangium affine (D.Don) Tzvelev Syn. Gnaphalium affine*
12. *Centella asiatica (L.) Urb.*
13. *Geranium nepalense Sweet*
14. *Sonchus sp*
15. *Galium aparine L.*
16. *Cardamine hirsute L.*
17. *Hypochaeris radicata L.*
18. *Rumex nepalensis Spreng.*
19. *Taraxacum officinale L.*
20. *Plantago major (L.) Weber ex F.H.Wigg.*
21. *Poa sp*
22. *Digitaria sp*
23. *Eriochlpa spp?*
24. *Eleusine spp*



Table 8: Pest list of cultivated of Chiretta, *Swertia chirayita* (Roxb.ex Fleming) H. Karst 2014/15

S.N.	Pest Category	Scientific Name of Pest	Common Name of Pest	Plant Parts Affected	Location
1	Fungi	<i>Fusarium solani</i>	Root rot	root	Maipokhari, Chamaita
2	Fungi	<i>Fusarium moniliforme</i>	Wilting	Leaf	Solbung, Maipatal

3	Fungi	<i>Fusarium oxysporium</i>	Rot and wilting	Root	solbung
4	Fungi	<i>Alternaria alternata</i>	Leaf spot	Leaf	Chaitgaun, Chamaita
5	Fungi	<i>Bipolaris sorokiniana</i>	Leaf blight	Leaf	Solbung, Maimajhuwa
6	Fungi	<i>Verticillium arbo-artum</i>	Wilt	Leaf, Stem	Chamaita ,Bagkhor
7	Fungi	<i>Colletotrichum gloeosporioides</i>		Leaf	Solbung Maipal
8	Fungi	<i>Trichoderma harzianum</i>		Stem	Solbung Maipal
9	Fungi	<i>Geotrichum candidum</i>		Leaf	Solbung Maipal
10	Bacteria	<i>Argobacterium tumefaciens</i>	Crown gall	Stem,	Solbung , Maipal
11	Fungi	<i>Verticillium sp.</i>		Leaf	Chamaita , Bagkhor
12	Insect	Stem borer 1		Leaf, shoot	
13	Insect	Stem borer 2		Leaf, shoot	

3.5 Findings of pest survey in Prickly Ash, *Zanthoxylum armatum* DC

The pest detection survey on Prickly Ash was conducted at Salyan district. During the first field visit, site selection work was done discussing with concerned stakeholders (DFO, DPRO, local traders, farmers). For this purposes, the procedure mentioned in “Technical Guidelines for Detection Survey of Plant Pests in Nepal” published by NPPO was followed. With all these background and discussions, the following fields were identified for the detection survey:

First Field

- Sall.P1 : Salam 6, Rim, Salyan
- Sall.P2 : Salam7, Rim, Salyan
- Sall.P3 : Sera 8, Kupinde, Salyan
- Sall.P4 : Damachaur 2, Mayani, Salyan
- Sall.P4 : Damachaur 4, Kalche, Salyan

Second Field

- Sal2.P1.SP1 : Salam 6, Rim, Salyan
Sal2.P1.SP2 : Salam 6, Rim, Salyan
Sal2.P1.SP8 : Salam 6, Rim, Salyan
Sal2.P2 : Salam 7, Rim, Salyan
Sal2.P3.SP1 : Kupinde 8, Maipokhari, Salyan
Sal2.P3.SP2 : Kupinde 8, Maipokhari, Salyan
Sal2.P3.SP3 : Kupinde 8, Maipokhari, Salyan
Sal2.P4.SP1 : Dhanbang 1, Khimchaur ,Salyan
Sal2.P4.SP2 : Dhanbang 1, Khimchaur ,Salyan

Third Field (storage)

- Sal3.P1 : Dhanbang 1, Khimchaur, Salyan
Sal3.P2 : Sarasawati Nagar, Shreenagar, Salyan
Sal3.P3 : Nokhi Ram, Rim 7, Salyan

3.5.1 Pathological Findings

Alternaria alternata (Fr.) Keissl. (Pleosporales: Pleosporaceae)

Sample number: Sal1.P1-S1 Sample number: Sal1.P2-S1
Isolate number: Za-Sal1-1-Le Isolate number: Za-Sal1-2-Le

This disease was collected from Rim and Rim 7 from first field visit. The disease specimen was collected from the leaf of the plant (Fig. 69). The brown spots at the leaf were observed .The further field information of *A. alternata* in Prickly Ash plant is described in pest data sheet (Annex XII).

The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.2 cm. Colony appear circular in form, entire margin with raised elevation, powdery to felty. The colony appear grey to olive green with white margin in obverse and appear dark brown black with light brown periphery.

Morphological characteristics:

The mycelium is sparse, greyish brown, fluffy, septate hyphae. The conidia variable in shape often ellipsoidal, obclavate or ovoid, pale to dark brown, 3-7 transverse septa, 1-several longitudinal septa (Plate 52). The size of conidium 28-58x10-25 μm . Conidium is polymorphous formed in branching chains.



Fig 69: The brown spots at the leaf



Plate 52: Microscopic view of *A. alternata*

***Alternaria radicina* Meier, Drechsler & E.D. Eddy (Pleosporales: Pleosporaceae)**

Sample number: Sal2.P1.SP2-S1

Isolate number: Za-Sal2-3-Le

This disease was collected from Rim 6 Salam during second visit. The disease specimen was collected from the leaf of the plant (Fig. 70). The brown spots and curling at the leaf were observed. The further field information of *A. radicina* in Prickly Ash plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth of fungus is 4.3 cm. The colony appears circular in form, flat elevation with entire margin. The colony of fungus appears brownish green at obverse and dark brown with light periphery at reverse.

Morphological characteristics:

The fungus produces grayish, fluffy mycelium in which black shiny conidia appears single or in pairs arranged in short chains. The conidia are variable in shape which is often ellipsoidal or may be obclavate or ovoid. Color of conidia is pale to dark brown with 3-7 transverse septa and one or several longitudinal or oblique septa which size is 27-57x9-27 μm (Plate 53).



Fig 70: The brown spots and curling at the leaf



Plate 53: Microscopic view of *A. radicina*

***Fusarium oxysporum* Schlecht.: Fr. (Hypocreales: Nectriaceae)**

Sample number: Sal2.P2.SP2-S1
Isolate number: Za-Sal2-4-Fr

Sample number: Sal2.P1.SP1-S1
Isolate number: Za-Sal2-5-Le

Sample number: Sal2.P1.S8-S8
Isolate number: Za-Sal2-6-Le

Sample number: Sal2.P4.S2-S1
Isolate number: Za-Sal2-7-Fr

Sample number: Sal3.P1
Isolate number: Za-Sal3-8-Fr

This disease was collected from Rim-7 Salam, Rim-6 Salam during second visit and from Dhanbang1, Khimchaur, Salyan district during second and third field visit. The disease specimen was collected from the fruit of the plant (Fig. 71) and leaf of the Prickly Ash plant. The white and greenish yellow growth on fruits was observed. The further field information of *F. oxysporum* in Prickly Ash plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.5 cm. Its colony is circular, floccose, crateriform in elevation with entire margin. The obverse and reverse view of fungus is pinkish white to purple .

Morphological characteristics:

The fungus produces aerial, septate and branched mycelium. The fungus micro conidia is oval to ellipsoidal which size ranges 5-12x2.5-3.5 μm while macro conidia is fusiform that is slightly curved with 3-5 septa that size ranges 24-45x3-5 μm (plate 54).



Fig 71: The white and greenish yellow growth on fruits



Plate 54: Microscopic view of *F. oxysporum*

***Fusarium dimerum* Penzig (Hypocreales:Nectriaceae)**

Sample number: Sal1.P3-S1 Sample number: Sal1.P4.SP2-S1

Isolate number: Za-Sal1-9-Le Isolate number: Za-Sal1-10-Le

This disease was collected from Kupinde-8, Sera and Damachaur-4, Kalche during first visit. The disease specimen was collected from the leaf of the plant (Fig. 72). The yellowish brown spots at the leaf were observed. The further field information of *F. dimerum* in Prickly Ash plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of the fungus is 2.7cm. The colony is floccose, circular in form, umbonate in elevation with entire margin. The fungus on obverse view appear white while in reverse appear light orange.

Morphological characteristics:

The fungus produces hyaline and septate mycelium. Its macro conidia is fusiform that is strongly curved with pointed apex. The fungus consists of 1-3 septa. Its size varies 10-20x3-4 μm . Micro conidia is absent (Plate 55)..



Fig 72: The yellowish brown spots at the leaf

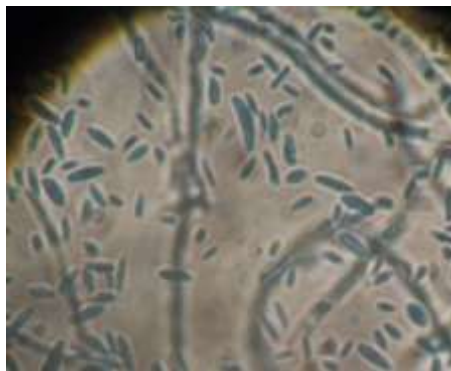


Plate 55: Microscopic view of *F. dimerum*

***Fusarium moniliforme* Sheldon (Hypocreales: Nectriaceae)**

Sample number: Sal1.P4.SP1-S1

Isolate number: Za-Sal1-11-Le

This disease was collected from Damachaur 2, Mayani Salyan during first visit. The disease specimen was collected from the leaf of the plant (Fig. 73a and 73b). The yellowish lesions all over the leaves were observed. The further field information of *F. moniliforme* in Prickly Ash plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 4.5 cm. The colony of fungus appears floccose which is circular in form. At obverse view of fungus is white while at reverse view of fungus is peach centre with white periphery.

Morphological characteristics:

The fungus produces hyaline, septate, sparsely branched mycelium. The micro conidia are hyaline, clavate bearing in long chain which size ranges 6-10x2-2.5 μm . The macro conidia is fusoid with 3-6 septa which size ranges 25-35x2.5-4 μm (Plate 56).



Fig 73a: The yellowish lesions all over the leaves

Fig 73b: The yellowish lesions all over the leaves

Plate 56: Microscopic view of *F. moniliforme*

Fusarium solani (Mart.) Sacc. (Hypocreales: Nectriaceae)

Sample number: Sal3.P2-S1
Isolate number: Za-Sal3-12-Fr

Sample number: Sal3.P3-S1
Isolate number: Za-Sal3-13-Fr

This disease was collected from storage of Saraswati Nagar, Shree Nagar, and Rim 7 of Salyan district. The disease specimen was collected from the fruit of the plant (Fig. 74). The whitish fungal out growth on fruits were observed. The further field information of *F. solani* in Prickly Ash plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 3.2 cm. The colony of fungus is regular, floccose with raised elevation. At obverse the fungus is white to cream while reverse view is yellowish with white periphery

Morphological characteristic:

The mycelium is hyaline, aerial and septate. The micro conidia is cylindrical to oval which size ranges 8-15x2-4 μm and macro conidia is inequilaterally fusoid with 1-5 septa which size ranges 35-55x4.6-6 μm (Plate 57)..



Fig 74: The whitish fungal out growth on fruits



Plate 57: Microscopic view of *F.solani*

Trichothecium roseum (Pers.) Link. (Hypocreales: Sordariomycetes)

Sample number: Sal2.P1-S1
Isolate number: Za-.Sal2-14-Le

Sample number: Sal2.P2-S1
Isolate number: Za-.Sal2-15-Le

Sample number: Sal2.P4-S1
Isolate number: Za-.Sal2-16-Le

Sample number: Sal2.P2-S8
Isolate number: Za-.Sal2-17-Le

Sample number: Sal2.P3-S1
Isolate number: Za-.Sal2-18-Le

This disease was collected from Rim 6 and Rim 7, Salam, Kupinde-8, Dhanbang-1, Khimchaur of second visit. The disease specimen was collected from the leaf of the plant (Fig. 75a and 75b). The black dots and mottling of leaf were observed. The further field information of *T. roseum* in Prickly Ash plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 3.4 cm. The colony is cottony, irregular in form with flat elevation. The obverse and reverse view of fungus is light peach with white periphery

Morphological characteristics:

The fungus produces hyaline mycelium which is septate. The conidium is oval, single septate and hyaline (Plate 58)..



Fig 75a: Black dots and mottling of leaf



Fig 75b: Black dots and mottling of leaf

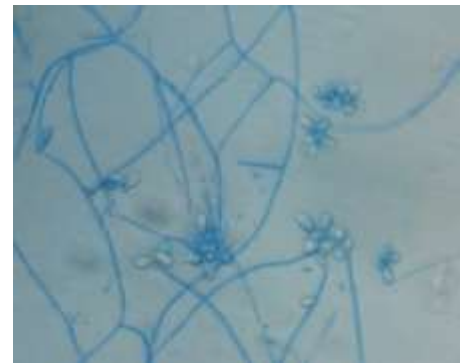


Plate 58: Microscopic view of *T. roseum*

Cladosporium sp. (Capnodiales :Davidiallaceae)

Sample number: Sal2.P1.SP2-S2

Isolate number: Za-Sal2-19-Le

This disease was collected from Rim 6, Salam, and Salyan during second field. The disease specimen was collected from the leaf of the plant (Fig. 76). The brown spots at the leaf were observed. The further field information of *Cladosporium* sp. In Prickly Ash plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The growth rate of fungus is 2.1 cm. The colony consists irregular in form, compact and with raised elevation. The colony of fungus at obverse appears army green and black at reverse

Morphological characteristics:

The fungus produces aerial mycelium with coiled hyphae. The conidia appear in 3-5 chain, shape ranges from ovate to cylindrical with olive brown color. The size of conidia ranges 3-4 μm (Plate 59).



Fig 76: Brown spots and curling of leaf



Plate 59: Microscopic view of *Cladosporium* sp.

***Xanthomonas campestris* (Pammel) Dowson (Xanthomonadales: Xanthomonaceae)**

Sample number: Sal2.P3-S2

Sample number: Sal2.P4.SP2-S2

Isolate number: Za-Sal2-20-Le

Isolate number: Za-Sal2-21-Le

This disease was collected from Kupinde 8, Sera, Salyan from first field visit. The disease specimen was collected from the leaf of the plant (Fig. 77). The yellowish brown spots at the leaf were observed. The further field information of *X. campestris* in Prickly Ash plant is described in pest data sheet (Annex XII). The cultural and morphological characteristics are:

Cultural characteristics:

The colony is moist, opaque, light yellow in color with smooth margin

Morphological characteristics:

The bacterium is Gram Negative which vegetative cell is straight rod arrange singly with rounded ends (Plate 60)..



Fig 77: Yellowish brown spots on leaf



Plate 60: Microscopic view of *X. campestris*

Candidatum *Liberibacter asiaticus* Jagoueix et. al (Rhizobiales: Rhizobiaceae)

Sample no.: Sal2.P2.S1 Sample no.: Sak2.P1.SP2

The diseased sample was collected from Rim 6 and Rim 7 of Salyan district during second field visit. The symptom of citrus hunaglongbing disease (greening) was observed on the leaf of Prickly Ash plant (Figure 78). The diseased plant consist mottling and immature fruit cracking types of symptoms. Most of the trees surveyed exhibited leaf yellowing symptoms like citrus greening diseases. The probability of this disease was more at these areas because of the availability of many citrus fruit species (Rutaceae family) and alternate host (*Murrya koenigii*). This evidence was further established with the presence of psyllids: *Diaphorina citri* (Insecta: Hemiptera: Psyllidae) at two of the surveyed areas.

The sample was submitted to National Academy of Science and Technology, Khumaltar, Kathmandu for further analysis (PCR) and confirmation of the bacterium.



Fig 78. Citrus greening of Prickly Ash Plant.

3.5.2 Entomological Findings

Black aphid, *Aphis fabae* (Scopoli); Aphididae: Homoptera: Hemiptera

General introduction of insects:

The black aphid *Aphis fabae* (Scopoli) was observed at almost all surveyed fields during first and second visit in Prickly Ash plants. This aphid, also called as black fly is a tiny black colored insect and usually feed in groups. This insect has been reported from different parts of the world and is polyphagous in nature, attacking many economical food and other crops. The field information of *A. fabae* in Prickly Ash plant is described in pest data sheet (Annex XII).

Visible damage symptoms in commodity pathway:

Both adult and nymph are the damaging stages, where the nymphs are observed feeding in groups. The aphid exists in winged and wingless forms, but in case of Prickly Ash plants we mainly observe the wingless form damaging the plant. Generally they suck plant sap from stems and leaves and cause distortion of the shoots, stunted plants, reduced yield and spoiled crops (Fig. 79). The aphid also acts as a vector for viruses that cause plant disease and the honeydew it secretes may encourage the growth of sooty mould.

During the first visit, the aphid infestation was observed at growing shoots and young leaves, while during the second visit they were concentrated at young leaves only. The insect was feeding from underside of the leaves in group. The infested leaves curl under and inward and become severely distorted. The leaf margin and sometimes the entire leaf become necrotic. These types of characteristic symptom were found more during second and third visit. The aphid damage was

observed in almost all plants in all survey sites. However their damage intensity was not affecting the whole tree that much.

Field identification of insects:

The black aphid (Plate 61), a member of the order Hemiptera is a small, soft-bodied insect that has specialised piercing and sucking mouthparts. At the field, the wingless form of aphid was seen in groups. The aphid was dull black in color with the body length of 1.5 to 2.5 mm. It has fairly short, dark, tapered cornicles/ siphunculi and a dark cauda. The femura bear fine, long hairs on all surfaces and the cauda has more than 10 hairs. The membranous wings of the alates are held angled over the body and the antenna was less than two-thirds of the length of the body.

Collection, transportation and preservation of the specimen:

The presence of aphid at the Prickly Ash plants during the observation was noticed by visual observation. The wingless form of black aphids was feeding at the tender shoot and the young leaves of the plant. The affected part of the plant along with the aphid was cut by secature. Then the aphids were collected by fine camel hair brush and transferred in 70% alcohol in screw capped vial. These vials were further wrapped by cotton roll and tightly placed inside the plastic bottle and transported to the DPR laboratory. At the laboratory, for identification purposes, the aphid specimen were cautiously taken out and placed at the blotting paper to absorb the alcohol. The remaining specimens were placed at 70% alcohol solution in 30 ml screw capped vials.



Fig 79: Aphid infestation damage on young leaves and shoot

Fig 80: Black aphids at the tender shoot of nepal pepper plant



Plate 61: Microscopic image of black aphid

Psyllid: *Diaphorina citri* (Kuwayama); Psyllidae: Hemiptera

General introduction of insects:

The psylla, *Diaphorina citri* (Kuwayama) was observed at Rim area (Sal2.P1.SP1 and Sal2.P2) during second and Dhanbang area (Sal3.P1) during third visit in Nepal pepper crop. This insect itself is a pest of minor importance but its economical importance is as the vector of very serious citrus huanglongbing disease (greening) caused by bacterium *Candidatus Liberibacter asiaticus* Jagoueix *et. al.* which is prevalent in Nepal. This insect has been reported from different parts of the world and mainly associated with the Rutaceae family plants. The field information of citrus psylla in Nepal pepper plant is described in pest data sheet (Annex).

Visible damage symptoms in commodity pathway:

The nymphs (Fig. 81) are the damaging stage and generally they are observed feeding in groups. During both field visits, we observed two to five nymph populations in one young leaf. The tip of the young leaves was twisted and some viscous solution was secreted where the nymphs were feeding (Fig. 82). The nymphs move in a slow and steady manner when disturbed. The adults of *D. citri* were mostly observed resting at the nearby host plants as, citrus, Prickly Ash, curry tree, etc (Fig 83).

The psylla damage resulted in defoliation and dieback. *D. citri* stunts and twists young shoots, so that the growing tips present a rosette appearance. Leaves are badly curled, and may be covered with honeydew and sooty mould; leaves drop prematurely. During our observation, its presence

was observed in some young leaves only, so the intensity of damage was not much. The important thing is, there are visible symptoms of greening disease (yet to be confirmed) at the leaves of Prickly Ash plant and also the vector (psylla) and hosts (many plants of Rutaceae family) are present around the periphery, which may be of serious concern to stakeholders.

Field identification of insects:

During our field visit, we observe the nymphs and adult stages of the psyllid. The nymphs were present in groups at the leaves folded at one side and the adults were observed resting at Nepal pepper and other plants of Rutaceae family. The adults generally rest on the terminal portion of plant, especially on the lower side of the leaves with their heads either pointing upward or downward to the leaf surface at an angle of 30°. When the adults are disturbed they readily take flight for a short distance.

The average size of mature nymphs is 1.6 mm long with light pink body and a pair of red compound eyes (Plate 62). The adults are about 2.5 mm long with yellowish brown body and greyish brown legs. The wings are transparent with white spots or light-brown with a broad longitudinal band in the center.

Collection, transportation and preservation of the specimen:

The presence of psylla at the Prickly Ash crop during the observation was noticed by visual observation. At first the adults were observed resting at curry tree plants, thereafter the nymphs were located feeding at the tender shoot and the young leaves of the plant.

The adults were collected by sweep net, killed in the killing jar and placed at screw capped vial with 70% alcohol. For the collection of nymphs, the affected part of the plant along with the insect was cut by secature. Then the psyllids were collected by fine camel hair brush and transferred in 70% alcohol in screw capped vial. These vials (containing adult and nymphs) were further wrapped by cotton roll and tightly placed inside the plastic bottle and transported to the DPR laboratory. At the laboratory, for identification purposes, the psyllid specimen were cautiously taken out and placed at the blotting paper to absorb the alcohol. The remaining specimens were placed at 70% alcohol solution in 30 ml screw capped vials.



Fig 81: Psyllid nymphs at the tip of Nepal pepper leaf

Fig 82: Damage by psyllid at Nepal pepper plant



Fig 83: Adult psyllid resting at Nepal pepper plant

Plate 62: Nymph of psyllid

3.5.3 Weed Data

As mentioned almost all Timur plants are cultivated at marginal lands; however the cropping pattern of some plants cultivation was followed as maize/soya bean – wheat/vegetables. The major associated species were *Quercus sp.*, *Rubus ellipticus Sm.*, *Lyonia ovalifolia (wall.) Drude*, *Prunus sp.*, *Alnus nepalensis D. Don.*, *Berberis aristata DC*, Bamboos, Oranges. Associated weeds are *Lantana camera L.*, *Ageratina adenophora(Spreng.) King & H. Rob.*, *Urtica dioica L.*, *Swertia angustifolia Buch-Ham ex D. Dom.*, *Salvia sp.* Grasses etc. Associated crop are maize, wheat, Green vegetables like Brassica spp.

In one site a parasitic plant was spotted arising from side twig of Timur tree. It was found to be *Scurulla parasitica*. Similarly some weed species were also found associated. For example, *Setaria sp.*, *Lantana camera*, *Swertia sp.*, *Eupatorium*, *Rubus Thalicttrum sp.*, *Berberis sp.* etc

Table 9: Pest list of cultivated Prickly Ash, *Zanthoxylum armatum* DC., 2014-15

SN	Pest category	Scientific Name	Common name of Pest	Plant part affected	location
1	Fungi	<i>Alternaria alternata</i> (Fr.) Keissl.	Leaf spot	Leaf	Rim 6, Rim 7- Salyan
2	Fungi	<i>Alternaria radicina</i> (Yoshii)	Leaf spot	Leaf	Rim 6- Salyan
3	Fungi	<i>Cladosporium</i> sp.		Leaf	Rim-6, Salyan
4	Fungi	<i>Fusarium oxysporum</i> Schlecht	Wilting of stem	Stem	Rim-6, Rim-7, Salyan
5	Fungi	<i>Fusarium solani</i> (Mart.) Sacc		Fruit	Saraswati Nagar, Shree Nagar, Rim 7 of Salyan
6	Fungi	<i>Fusarium moniliforme</i> Sheldon		Leaf	Damachaur2, Mayani Salyan
7	Fungi	<i>Fusarium dimerum</i> Penzig		Leaf	Kupinde8, Sera and Damachaur 4, Kalche
8	Fungi	<i>Tricothecium roseum</i> Link.		Leaf	Rim 6, Rim 7, Salam, Kupinde-8, Dhanbang 1, Khimchaur
9	Bacteria	<i>Xanthomonas campestris</i> (Pammel) Dowson	Wilt	Leaf	Kupinde-8, Sera Salyan
	Insect	<i>Aphis fabae</i> (Scopoli)	Black aphid		
	Insect	<i>Diaphorina citri</i> (Kuwayama)	Psyllid		

CHAPTER IV: CONCLUSION AND WAY FORWARD

This is the first and foremost structured survey program (although it was a small project in terms of the work to be done) to create an authentic pest database of MAPs in Nepal. The overall objective of the project was to support the export of MAP commodities. Generating information to undertake Pest Risk Analysis with the help of field based pest surveys is expected to help increase the export. The project was just for pest detection and only detection of the pests would not totally be able to create the database for using in PRA. As this is the good start for generating pest database, some recommendations for further works so as to create detailed pest record is sought imperative.

National pest database should include the pest record of specific commodity in the major production areas of the country. Extensive coverage of several production areas by the survey is the best method to generate detailed pest database. This survey covered only few selected VDCs of a particular district for each commodity. For example, Wild Asparagus of Makawanpur district was selected for survey of its pests. Similarly, only Ilam district was purposively selected for pest survey in Chiraito. So, other production areas except surveyed district are not represented in this report. For example, the pests of Prickly Ash found in Surkhet and Dang district might not be the same as what this survey found in Salyan district while surveying.

As stated in the limitations of the study, the database generated and presented in this report is just a year index of the pest status of limited areas i.e. this study does not intends to prepare the national pest database. The pests found in a commodity growing in one location may differ from those found in another location. This happens because of the variations in micro-climatic features, altitude and the nature of pest. Normally long term surveillance of pests attacking any commodity in a given location can generate more real picture of pest status. Some pests are highly sensitive to micro-climate and may appear erratically in different years. Sporadic appearance of pests in different years has been observed in several commodities in several locations. Sporadic pests can be picked up as live specimen, provided that the survey is regular activity for several years. Because, the pests found in one year may not appear during next year survey and the pest not observed during one year might be appearing in next year survey. Hence, in order to prepare the completed national pest list, it can be recommended that the survey program must be ongoing and must cover extensive production areas. During the entire course of this survey, few of the

devastating pests attacking severely were not caught up properly. The peak pest activity and the field survey schedule were not matched up in some cases. For example, the survey did not find any insect specimens of stem borer in Chireeta, although the attack was severe and the symptoms were intense. In this case the surveyors were able to collect only the damaged parts of the host but not the pest intact. Similar was the case in aphids of Prickly Ash, in this case, peak activity of aphids were not observed but the damage was found severe. Likewise, the fruit and stem borer in Soapnut plants were not observed during the first field visit, but their symptom and damage could only be collected during the consecutive field visits. The survey plan was not properly fitted with the few pests' peak activities, and this happens usually with short duration survey programs. This necessitates further extended survey project.

Multiple institutional collaboration is needed to prepare the pest data base. Institutionalized involvement of NARC for generating database on different spheres of pest bio-dynamics is important. NARC is an authentic research organization with minimum scientific infrastructure and scientific manpower to study about plant pests. The team paid effort to involve NARC as a partner institution for pest survey but was not materialized. NARC do have good logistic background to maintain the pest library and reference laboratory, although it needs some financial and technical for adjusting entirely new activities like this survey within its institutional framework. NARC should be involved for conducting rest of the activities of this survey related to diagnostics, reference laboratory and pest library.

Orientation training followed by pilot study for the field technician in different aspects of field survey is seen essential. Despite the efforts, the team was unable to carry out pilot study before allowing the field technicians to go for field survey; this led to few of the operational ambiguities. For example, there were a lot of variations in filling up of field survey data sheets. Uniformity in filling up of forms is needed for easier analysis of the collected data. In this connection, development of specific protocol for field coding system for collected sample is sought needed for future. Similar coding system needs to be developed for the reference cultures and the specimen in the pest libraries as well.

While undertaking PRA by the importing countries, the exporting country must supply the information as needed to them. The information is asked through PRA request form and the request sent to the NPPO. The information asked by the importing country must be generated with the

help of ongoing surveillance program. Ongoing surveillance is needed for ensuring the exactness of the survey findings extracted previously. It also helps to complete the data shortage like that of "adult specimen of stem borer" of *Chireeta* in the current project.

Few of the researchable issues may arise while completing the pest record. Involvement of NARC along with the current partners collaboratively can settle these issues. While conducting PRA by the importing partners, they might ask for qualitative or quantitative database of individual pests those are of researchable in nature. The research activities might be on: I) the study of pest's rate of reproduction and spread, II) study of vectors, III) study on reproductive strategy of pest, IV) study on genetic variability and adoptability, V) finding out the minimum population needed for establishment, VI) study of human activities that critically affect on pest's biology and spread, VII) study on relation of pest survival with different crop practices like irrigation, soil conservation practice, manuring, training, pruning, harvesting, processing, pesticide application, use of natural enemies etc. VIII) study on related alternate and collateral hosts, etc. Similarly, different aspects of pest management practices like finding out the best method of inspection for particular pest, developing testing and diagnostic protocol for specific pest, development of protocol on farm certification schemes, study on chemical treatment options, finding the economic threshold level of the pest, study of the pest with the nature of latent infection, study to know the best treatment option before shipment of the consignment, extensive surveys to determining the concentration of pest in different areas of production, studies on possibility to declare the pest free areas or area of low pest prevalence, etc. might also needs to be researched.

Quantitative aspects of pest's impact in the production areas are also of equal importance. Monitoring surveys are generally recommended to generate the quantitative data related to economic impact of pest. Detailed pest record to be used in PRA commonly demands for monitoring studies. The area of further activities can be the I) assessment of crop loss in term of quality and quantity, II) assessment of the amount and frequency of damage caused by particular pest, III) assessment of prevailing commercial procedure for consignment preparation and correlation with pest survival, IV) identification of most susceptible species, V) assessment of revenue loss due to pest attack, VI) studies on adoptability and virulence of pest that affects damage, VII) cost benefit analysis for assessing efficacy of control measures applied, VIII) studies of the effects on keystone species, IX) study on ecological and environmental significance of pests, etc.

AS stated earlier, there are several things to be addressed in the upcoming survey programs, additional important recommendations are experts must be registered in the specific roster as designed by NPPO, better incentives for laboratory technicians and field experts should be managed, and the length of the field visits should be extended.

Finally and more importantly, Plant Protection Regulation has assigned NPPO to take a lead role for carrying out any type of survey and surveillance function. This survey was conducted by taking the due permission of NPPO, following the NPPO's approved guidelines (NPPO, 2014). So, NPPO must co-ordinate the stakeholders including current partners to ensure that the quality of survey was maintained. Further, NPPO must be accountable for the findings of survey program conducted within the country. As NPPO is responsible for bilateral dealings for market access in any importing country, it has to take the ownership of the reports and findings of this project. Also, NPPO must take its hold to ensure that the collected pest species are well preserved, their identification is verified, specimen are maintained in the pest library and the database is updated and owned by its central database management system.

GLOSSARY

Additional declaration	An additional statement that is required by an importing country to be described on a Phytosanitary Certificate and which provides specific additional information of a consignment in relation to regulated pests or regulated articles.
Buffer zone	An area surrounding or adjacent to an area officially delimited for Phytosanitary Purposes in order to minimize the probability of spread of the target pest into or out of the delimited area, and subject to Phytosanitary or other control measures, if appropriate [ISPM 10:1999; revised ISPM 22:2005]
Commodity	A type of plant, plant product, or other article being moved for trade or other purpose [FAO, 1990; revised ICPM, 2001]
Commodity pest list	A list of pests present in an area which may be associated with a specific commodity [CEPM, 1996]
Consignment	A quantity of plants, plant products or other articles being moved from one country to another and covered, when required, by a single Phytosanitary Certificate (a consignment may be composed of one or more commodities or lots) [FAO, 1990; revised ICPM, 2001]
Contamination	Presence in a commodity, storage place, conveyance or container, of pests or other regulated articles, not constituting an infestation [CEPM, 1996]
Control (of a pest)	Suppression, containment or eradication of a pest population [FAO, 1995]
Establishment (of a pest)	Perpetuation, for the foreseeable future, of a pest within an area after entry [FAO, 1990; revised FAO, 1995; IPPC, 1997; formerly established]
Field	A plot of land with defined boundaries within a place of production on which a commodity is grown [FAO, 1990]
Habitat	Part of an ecosystem with conditions in which an organism is naturally present or can establish [ICPM, 2005]
Harmonization	The establishment, recognition and application by different countries of Phytosanitary Measures based on common standards

	[FAO, 1995; revised CEPM, 1999; based on the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (WTO, 1994)]
Host pest list	A list of pests that infest a plant species, globally or in an area [CEPM, 1996; revised CEPM, 1999]
Host range	Species capable, under natural conditions, of sustaining a specific pest or other organism [FAO, 1990; revised ISPM 3:2005]
Import permit	Official document authorizing importation of a commodity in accordance with specified Phytosanitary import requirements [FAO, 1990; revised FAO, 1995; ICPM, 2005]
Infestation (of a commodity)	Presence in a commodity of a living pest of the plant or plant product concerned. Infestation includes infection [revised CEPM, 1999]
Inspection	Official examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with Phytosanitary Regulations [FAO, 1990; revised FAO, 1995; formerly inspect]
International Standard for Phytosanitary Measures	An international standard adopted by the Conference of FAO, the Interim Commission on Phytosanitary Measures or the Commission on Phytosanitary Measures, established under the IPPC [CEPM, 1996; revised CEPM, 1999]
Introduction (of a pest)	The entry of a pest resulting in its establishment [FAO, 1990; revised FAO, 1995]
Monitoring	An official ongoing process to verify Phytosanitary situations [CEPM, 1996]
Monitoring survey	Ongoing survey to verify the characteristics of a pest population [FAO, 1995]
National plant protection organization	Official service established by a government to discharge the functions specified by the IPPC [FAO, 1990; formerly plant protection organization (national)]

Natural enemy	An organism which lives at the expense of another organism in its area of origin and which may help to limit the population of that organism. This includes parasitoids, parasites, predators, phytophagous organisms and pathogens [revised ISPM 3:2005]
Pathogen	Any agent causing disease. This includes fungi, bacteria, viruses, nematodes, mycoplasma, viroid and others
Pest	Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products or beneficial organisms. Plant pest is sometimes used for the term pest.
Pest diagnosis	The process of detection and identification of a pest [ISPM 27:2006]
Pest record	A specimen based document providing information concerning the specific pest at a particular location at a certain time, within an area (usually a country) under described circumstances [ISPM 6. 1997]
Pest risk analysis	The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any Phytosanitary measures to be taken against it [ISPM 2:2007]
Pest status	Presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgment on the basis of current and historical pest records and other information [revised ICPM, 1998]
Phytosanitary certificate	An official paper document or its official electronic equivalent, consistent with the model certificates of the IPPC, attesting that a consignment meets Phytosanitary import requirements [FAO, 1990; revised CPM, 2012]
Phytosanitary measure	Any legislation, regulation or official procedure having the purpose to prevent the introduction or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests [FAO, 1995; ICPM, 2002]
Phytosanitary procedure	Any official method for implementing Phytosanitary Measures including the performance of inspections, tests, surveillance or treatments in connection with regulated pests [FAO, 1990; revised

FAO, 1995; CEPM, 1999; ICPM, 2001; ICPM, 2005]

Phytosanitary

regulation

Official rule to prevent the introduction or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests, including establishment of procedures for Phytosanitary Certification [FAO, 1990; revised FAO, 1995; CEPM, 1999; ICPM, 2001]

Phytosanitary Security

(of a consignment)

Maintenance of the integrity of a consignment and prevention of its infestation and contamination by regulated pests, through the application of appropriate Phytosanitary Measures [CPM, 2009]

Plant product

any plant product not manufactured for use (including feeds) or any manufactured product that may, by the reason of nature of their processing or otherwise, create a risk for the introduction, establishment and spread of pests. (NG)

Plant quarantine

All activities designed to prevent the introduction or spread of quarantine pests or to ensure their official control [FAO, 1990; revised FAO, 1995]

Plants

Living plants and parts thereof, including seeds and germplasm [FAO, 1990]

Production site

A defined part of a place of production, that is managed as separate for Phytosanitary purpose

Quarantine

Official confinement of any plants, plant products or other articles in the specified place prescribed by the inspector for the purpose of observing, testing, investigating, inspecting and examining to know whether such plants, plant products or other articles contain any pest or if they are healthy or not and treating them if they are found not to be healthy (NG-modified?)

Quarantine pest

A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled [FAO, 1990; revised FAO, 1995]

Reference specimen	Specimen, from a population of a specific organism, conserved and accessible for the purpose of identification, verification or comparison, maintained in the pest record [ISPM 3:2005; revised CPM, 2009]
Spread(of a pest)	Expansion of the geographical distribution of a pest within an area [FAO, 1995]
Standard	Document established by consensus and approved by a recognized body, that provides, for common and repeated use, rules, guidelines or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context [FAO, 1995; ISO/IEC Guide 2:1991 definition]
Stored product	Unmanufactured plant product intended for consumption or processing, stored in a dried form (this includes in particular grain and dried fruits and vegetables) [FAO, 1990]
Surveillance	An official process which collects and records data on pest occurrence or absence by survey, monitoring or other procedures [CEPM, 1996]
Survey	An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species are present in an area [FAO, 1990; revised CEPM, 1996]
Test	Official examination, other than visual, to determine if pests are present or to identify pests [FAO, 1990]

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