

**STUDIES ON DOWNY MILDEW OF ONION  
(*Allium cepa* L.)**

*Thesis*

**by**

**ANKITA  
(H-2016-70-M)**

**Submitted to**



**Dr YASHWANT SINGH PARMAR UNIVERSITY  
OF HORTICULTURE AND FORESTRY  
SOLAN (NAUNI) HP - 173 230 INDIA**

**in**

**Partial fulfilment of the requirements for the degree**

**of**

**MASTER OF SCIENCE  
(AGRICULTURE)  
PLANT PATHOLOGY  
DEPARTMENT OF PLANT PATHOLOGY**

**2018**

**Dr. Sunita Chandel**  
Professor

Department of Plant Pathology  
Dr. Yashwant Singh Parmar University of  
Horticulture and Forestry  
(Nauni) Solan (HP) - 173 230 India

### **CERTIFICATE-I**

This is to certify that the thesis titled, “**Studies on downy mildew of onion (*Allium cepa* L.)**”, submitted in partial fulfillment of the requirements for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY** in the discipline of **PLANT PROTECTION** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) – 173230 is a bonafide research work carried out by **Ms. ANKITA (H-2016-70-M)** daughter of Shri Sunil Kumar under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

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


**Place: Nauni, Solan**  
**Date :**


---


**(Dr. Sunita Chandel)**  
**Chairman**  
**Advisory Committee**

## CERTIFICATE-II



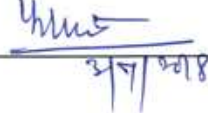
This is to certify that the thesis titled, "Studies on downy mildew of onion (*Allium cepa* L.), submitted by Ms. Ankita (H-2016-70-M) daughter of Shri Sunil Kumar to Dr. Yashwant Singh Parmar University of Horticulture and Forestry (Nauni) Solan (HP) - 173 230, India in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in the discipline of **PLANT PROTECTION** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.


  
Dr Sunita Chandel  
Chairman  
Advisory Committee

  
Dr Sanjeev Sharma  
Principal Scientist  
External Examiner

  
Dr Harish Kumar Sharma  
Principal Scientist  
Dean's Nominee

### Advisory Committee

1. Dr. H.R. Gautam  
Principal Scientist, Plant Pathology 
2. Dr. D.K. Mehta  
Senior Scientist, Vegetable Science 
3. Dr. K.C. Sharma  
Principal Scientist, Entomology 

  
Dr A K Gupta  
Professor and Head  
Department of Plant Pathology

Dean  
College of Horticulture  
Dr YSPUHF Nauni Solan, (HP)

# ACKNOWLEDGEMENT

---

---

*Putting all the things aside first of all, I would like to thank “God -The Almighty” for providing me the gracious gifts of all strength with patience and courage bestowed upon me to overcome various challenges to cross important milestone of my assigned profession. The thesis appears in its current form due to the assistance and guidance of several people. I would like to offer my sincere thanks to all of them.*

*At the very outset, I express my heartiest veneration and heartfelt gratitude to my respected and esteemed teacher and chairperson of my advisory committee, **Dr. Sunita Chandel**, Professor, Department of Plant Pathology for her valuable and express guidance, timely suggestions, close counsel critical evaluation, everlasting patience, and constant encouragement at every step of research work and finalization of this manuscript. It was a great opportunity for me to work under her guidance.*

*I extend my heartiest thanks to **Dr. AK Gupta**, Professor and Head, Department of Plant Pathology, members of my Advisory Committee, **Dr. HR Gautam**, Principal Scientist, Department of Plant Pathology; **Dr. DK Mehta**, Senior scientist, Department of Vegetable Science; **Dr. KC Sharma**, Principal Scientist, Department of Entomology; for their valuable suggestions and guidance during the investigation and manuscript preparation. I would also like to thank **Dr. Rajender Sharma**, Scientist, Department of Seed Science and Technology who allowed me to take up my research work at the Farm.*

*Every effort is motivated by an ambition and all ambitions have inspirations behind. I owe this pride place to my ever loving Papa **Shri Sunil Kumar**, Mummy **Smt. Anita Kumari**, sisters **Shweta and Nivedita**, brother **Udit**, all elders in my family for their oceans of love and moral support bestowed upon me which steered the life of this cipher to the present shape.*

*“A friend is one whom you can pour your heart out”. I find lacunae of words to express thanks, gratitude, affection and love plied by these pearls. It brings immense joy, full of tears rolling out of my eyes that these God sent angels always stood by me during every season of life. From the core of my heart I would like to convey my sincere thanks to my all near and dear friends especially **Vijay Kamal, Gurwinder, Humendra, Abhilasha, Chidambara, Vinay, Maddy, Reshal, Jyotasaana, Rishika, Sonakshi, Deepika, Pooja and Naveen** for their cooperation, valuable help and jovial company.*

*I would like to mention my sincere thanks and co-operation to my seniors viz, **Vijay Sir, Adikshita di, Gurvinder Sir, Praneet Sir, Sumit Sir, Bhagta sir, Bharti Mam** for their help and support. I would also like to mention my sincere thanks and co-operation to my juniors **Shivani, Sakshi, Ashwini and Pullar Reddy**.*

*Though acknowledging is an endless task. I would finally like to express my sincere gratitude and thanks to all those who I may have inadvertently failed to mention here. The help of all whose contribution, big or small is their towards the completion of this manuscript is gratefully acknowledged.*

*Lastly thanks to all beloved and respected people around me who directly and indirectly helped me.  
.....Any omission in this acknowledgement does not mean lack of gratitude.*

**Place: Nauni-Solan**

**(Ankita)**

**Date:**

## CONTENTS

<b>Chapter</b>	<b>Title</b>	<b>Page</b>
<b>1</b>	<b>INTRODUCTION</b>	<b>1-3</b>
<b>2</b>	<b>REVIEW OF LITERATURE</b>	<b>4-16</b>
<b>3</b>	<b>MATERIALS AND METHODS</b>	<b>17-23</b>
<b>4</b>	<b>RESULTS AND DISCUSSION</b>	<b>24-41</b>
<b>5</b>	<b>SUMMARY AND CONCLUSION</b>	<b>42-44</b>
	<b>LITERATURE CITED</b>	<b>45-55</b>
	<b>ABSTRACT</b>	<b>56</b>
	<b>APPENDICES</b>	<b>i-iii</b>
	<b>BRIEF BIO-DATA</b>	

## LIST OF ABBREVIATIONS

---

%	:	Per cent
/	:	Per
°C	:	Degree Celsius
CD	:	Critical difference
cm	:	Centimeter
BOD	:	Biological Oxygen Demand
<i>et al.</i>	:	et alia (Co – workers)
etc.	:	etcetera (and rest)
±	:	Plus or minus
C	:	Control
g	:	Gram
H.P.	:	Himachal Pradesh
hr	:	Hour
<i>i.e.</i>	:	Id Est (that is)
<i>viz.</i>	:	Vide licet (namely)
ml	:	Milliliter
mg	:	Milligram
min	:	Minute
mm	:	Millimeter
MSS	:	Mean sum of square
WG	:	Wettable Granules
no.	:	Number
sec	:	Second
sp.	:	Species
EC	:	Emulsifiable concentrates
WP	:	Wettable powder
PDI	:	Per cent Disease Incidence
>	:	More than
µg	:	Micrograms
SS	:	Sum of square
RH	:	Relative humidity
ppm	:	Parts per million

---

## LIST OF TABLES

Table	Title	Page(s)
4.1	Incidence and severity of onion downy mildew in Himachal Pradesh during 2017-18	24
4.2	Morphological characters of causal organism ( <i>Peronospora destructor</i> (Berk.) Casp. ex Berk)	26
4.3	Pathogenicity of <i>P. destructor</i> causing downy mildew of onion	27
4.4	Effect of different temperature regimes on sporangial germination and germ tube length of <i>Peronospora destructor</i>	29
4.5	Effect of different relative humidity levels on sporangial germination and germ tube length of <i>Peronospora destructor</i>	30
4.6	Effect of meteorological factors on the development of downy mildew of onion during 2017-18	32
4.7	Simple correlation coefficients between per cent disease index (PDI) and meteorological factors	33
4.8	Partial correlation coefficients between per cent disease index (PDI) and meteorological factors	34
4.9	Multiple regression equation and coefficient of determination of disease development based on meteorological factors	34
4.10	Effect of different fungicides on sporangial germination inhibition (%) of <i>Peronospora destructor</i>	37
4.11	Effect of different fungicides on sporangial germ tube length ( $\mu\text{m}$ ) of <i>Peronospora destructor</i>	38
4.12	Evaluation of SAR chemicals and fungicides as foliar sprays against onion downy mildew under field conditions	40

## LIST OF PLATES

Plate	Title	Between Pages
1.	Symptomatology of downy mildew on onion	25-26
2.	Morphological identification of downy mildew pathogen	25-26
3.	Pathogenicity symptoms produced by downy mildew pathogen (Spray and drop inoculation method)	27-28
4.	Pathogenicity symptoms produced by downy mildew pathogen (Detached leaf method)	27-28
5.	Effect of fungicide on sporangial germination and germ tube length production under <i>in vitro</i>	39-40
6.	<i>In vivo</i> evaluation of SAR chemicals and fungicides	39-40

## LIST OF FIGURES

Figure	Title	Between Pages
1.	Graphical representation of different meteorological parameters on downy mildew of onion (cv. Palam Lohit) under field conditions.	33-34

## *Chapter-1*

# INTRODUCTION

---

Onion (*Allium cepa* L.) is a biennial vegetable grown as an annual and used for enriching the food. It is placed in the family *Amaryllidaceae* having chromosome number,  $2n=16$  (Anonymous, 1999). It is likewise a rich source of vitamin C, phosphorus, calcium and carbohydrates and its pungency is because of a volatile oil known as Allyl-propyl disulphide. In spite of the fact that there are numerous substitutes to onion like garlic (*Allium sativum* L.), shallots (*Allium ascalonicum* L.) and leeks (*Allium porrum* L.), the production of the last three is immaterial compared to the previous, because of the popularity of onion in making stews, soups and numerous different recipes (Anonymous, 1982).

Onion is an important source of vegetable and its seeds are valued as one of the condiments (Baswana, 1984). It is one of the critical condiments consumed as a seasoning agent all the year round, however the green leaves, younger and even developed bulbs are eaten raw or utilized for preparation of vegetables. Even the little bulbs can be pickled in vinegar. The onion has been cultivated for more than five thousand years and has been utilized as an important constituent of herbal medicine. The recent research has revealed that onions if eaten routinely may have an influence in counteracting heart disease and other different ailments (Anonymous, 1999).

The name Onion is derived from the Latin, *unio*, signifying "one huge pearl", and it is fascinating to note that the Chinese called the Onion as the "gem among vegetable" (Anonymous, 2015). It is now cultivated all through the world having originated in temperate and has been bred to adjust to the tropics also (Ross, 2001).

*Allium* is the genus of onion with around 1250 types of perennial bulbous plants. Onions are among the world's most seasoned cultivated plants. They were most likely known in India, China, and the Middle East before written history. It is considered as native to South Western Asia yet is presently grown all through the world, predominantly

in the temperate locales. Although the Central Asiatic countries in the area of Iran and Pakistan are considered as the primary centre of origin of onion. The close East Asiatic and Mediterranean locales are considered to be the secondary centers of origin (Anonymous, 2004). They are developed in every temperate area of the world, particularly in Egypt, Spain, Italy and the United States (Jordan, 1915).

Onion (*Allium cepa* L.) is an economic vegetable crop not just for domestic consumption yet in addition as one of the highest foreign exchange earner among the other agricultural products. The major onion growing countries are China, India, USA, Iran and Russian Federation (FAO, 2016). In India the major onion producing states are Maharashtra, Gujarat, Odisha, Karnataka, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Bihar, Punjab and Rajasthan (NHB, 2017). It occupies an area of 1306 thousand ha, with production of 22427 thousand metric tonnes in India (NHB, 2017). The export of onion amid 2016-17 was 24,15,757.11 MT to the world for the worth of Rs. 3,106.50 crores/ 464.02 USD Millions (APEDA, 2017). India is the second biggest producer of onion, in the world next just to China, however the productivity of onion in India is quite low i.e. 14.21 tonnes/ha as compared to China and other countries like Egypt, Netherlands, and Iran etc.

In Himachal Pradesh, onion is grown in subtropical and dry temperate zone. It is not the commercial crop of Himachal Pradesh but has the potential to become one. It occupies an area of about 2530 hectares with total annual production of about 47960 metric tonnes and has productivity of 18.96 metric tonnes per hectare (PDOGR, 2016). In spite of favourable environmental conditions for onion cultivation, the production of crop is affected by various insect pests, nutritional disorders and diseases. Among the important diseases responsible for creating a gap in the production level, incidence of downy mildew, purple blotch, Stemphylium blight and smudge are most prominent and widely distributed in the entire area. One of the main economically important onion disease is downy mildew caused by *Peronospora destructor* (Berk.) Casp. ex Berk. (Yarwood, 1943). It is cosmopolitan in distribution and yield loss assessment records showed that it can reduce yield by as much as 15-27 per cent in bulbs and 46-100 per cent in seed crops (Jones, 1932).

The disease was first time reported in England in 1841. Since then it has spread to all parts of the world, where onions are grown (Sherf and Macnab, 1986). In India, the pathogen was recorded for the first time during 1974-75 and 1975-76 from Kashmir valley where it caused 60-100% losses in crop field (Mirakhur *et al.*, 1977). Subsequently the disease was reported from Kurukshetra in Haryana state (Gupta *et al.*, 1984).

The disease is characterised initially by slightly pale spots which later developed into a light brown or purplish shade. As spots enlarge, often girdling of the leaves and a greyish violet furry mould develops on the spot's surface. Plants may get dwarfed, distorted, pale and slightly green. Plants often are not killed, but bulb quality is poor and often spongy. Lesions weaken the seed stems that lead to breakage, causing seed to shrivel (Cynthia and Ocamb, 2008). Successive leaves are attacked leaving the youngest one. The bulbs of such plants are unable to develop fully and remains immature (Chupp and Sherf, 1960). Onion downy mildew occurs regularly in a moderate to severe form in Himachal Pradesh ever since its breakout was first reported in the state (Singh *et al.*, 1987) and causes 12-75% losses in bulb yield (Sugha and Singh, 1991).

Downy mildew is one of the serious diseases on onion which occurs in moderate to severe form depending on the prevailing environmental conditions in HP. Therefore, looking into the economic significance of the crop and high magnitude of crop loss due to high intensity of the pathogen in Himachal Pradesh, the present investigation has been proposed on studies on downy mildew of onion with the following objectives:

1. To assess the disease status in Solan district (HP) and to identify the pathogen.
2. To study the environmental factors on disease development.
3. To evaluate the potential of fungicides and SAR inducing chemicals in controlling downy mildew of onion.

## *Chapter-2*

# REVIEW OF LITERATURE

---

The available pertinent literature on following aspects of onion downy mildew is reviewed as under:

### **2.1 OCCURRENCE**

Downy mildew of onion, caused by the fungus *Peronospora destructor* (Berk.) Casp. ex Berk, is worldwide in presence and makes immense financial misfortunes to the crop (Cook, 1932; Viranyi, 1974). The pathogen attacks diverse kinds of onion, however, is especially damaging to the common onion, i.e. *Allium cepa*. It is predominant in occurrence, in most onion producing territory all around the world, causing greater loss in both yield and quality (Lorbeer and Andaloro, 1984; Schwartz and Mohan, 1995). In India it was reported for the first time in 1974 from Kashmir valley (Mirakhur *et al.*, 1977).

The disease is caused by *Peronospora* species and reported from northern hilly track and fields especially in high humid areas. The disease is most exceedingly terrible in damp conditions and late planting of the crop, as well as utilization of higher doses of fertilizers and higher number of irrigation increases the disease severity (NHB, 2017).

Disease can be disseminated by the wind and contaminated seeds. The congenial environment for disease is humid and cool conditions. Amid the period of strong winds, prevention and control measures are necessary (Arita and Santos, 2012). The pathogen attack is seen in all the phenological stages of the crop, the most elevated estimations of severity of the infection correspond with low temperatures (6-20° C), frequent rainfall or relative humidity more than 95 per cent. As indicated by Hildebrand and Sutton (1982, 1984) no pathogen attacks when relative humidity is under 90 per cent and temperature regimes higher than 24° C since they impact the development and sporulation of the pathogen adversely.

### **2.2 LOSSES**

If there is an occurrence of downy mildew in green onions, yield losses can be as high as 100 per cent, with the whole crop being disposed off as downy mildew infections

make them unmarketable. Losses to seed production are often caused by the collapse of contaminated seed stalks and poor germination of seeds gathered from infected stalks (Schwartz and Mohan, 1995). The disease can cause serious losses in short span of time frame in the midst of cool and humid climatic conditions (Hoffmann *et al.*, 1996). Actual yield losses of 60 to 75 per cent in bulb onions have been recorded (Develash and Sugha, 1997 a).

Yield losses up to 75.11 per cent accomplished because of downy mildew as per Jayakumar *et al.* (2008). These losses, basically result from serious infections in onion bulbs, causing early defoliation, diminished bulb sizes, moreover, poor storage quality of bulbs (Surviliene *et al.*, 2008). Relative humidity up to 100 per cent and temperatures stretch out from 20 to 28°C are perfect for downy mildew disease with purple blotch (Kumar, 2007).

The losses generally attributed from serious infections in bulb onion crops causing early defoliation, diminished bulb sizes and poor storage quality of bulbs (Lorbeer and Andaloro, 1984; Gilles *et al.*, 2004; Gianessi and Reigner, 2005). Onion downy mildew has widespread occurrence and can affect whole onion leaf area, causing severe reduction in the bulb growth (Scholten *et al.*, 2007).

### **2.3 SYMPTOMATOLOGY**

The fungus may fruit on any part of the seed plants, yet the fruiting structures normally seen first on the leaves and after that on the seed-stalks. They may occur anytime on the seed-stalk from the base to the top and occasionally are found on the inflorescence. Soon after the fungus has fruited, the epidermal and palisade cells of the affected parts collapse and the resulting lesion become white, slightly sunken and roughened. The lesions are typically circular or elliptical in shape and involve just a single side of the stalk. At times, in any case, they are vast and girdle the stalk. Lesions are fundamentally the same as in appearance with the mechanical injury caused by two stalks rubbing together (Cook, 1932). On the off chance that leaf damage is severe, bulb development is particularly hindered and as a result a substantial number of "bottlenecked" onion bulbs develop (Rondomanski, 1967).

Butler and Jones (1955) revealed that the disease attacks the plants at all stages of growth and all parts of the plant might be invaded. The disease occurs in two stages: the

primary stage (when infected bulb is planted) and the secondary stage (when a healthy plant at leaf stage becomes infected from spores produced by primary stage). The most characteristic feature of onion downy mildew is the development of grey “downy” growth on the leaves under humid conditions, hence the name. This superficial growth which consists of sporangiophores and sporangia of the fungus emerges from the sub-stomatal air spaces in the leaf tissue (Wheeler, 1972). Symptoms often seen first on older leaves. If weather conditions are moist and temperatures are low, masses of dark to violet parasitic spores conceal infected leaves, which become girdled, collapse and may die. The dead leaf tissue is quickly colonized by purple blotch, which is dull in colour and obscures downy mildew.

Plants raised from infected bulbs are infected systemically. These plants remain stunted, distorted and light green in colour, causes shrivelling and softening during storage. Under humid weather conditions, the greyish violet downy growth of the fungus can be seen on the whole leaf surface. Local infection is caused via air-borne conidia which produce oval to cylindrical spots. Lesions look violet to purple and might be confused with initial lesions of purple blotch. In the field, vast yellowing areas that extends out in the direction of the prevailing winds are an indication of downy mildew. Affected plants might be dwarfed, leaves distorted and may produce bulbs of reduced size and quality (Schultz and French, 2009).

Older leaves are attacked first and infection spreads to the sheath. Affected leaves become light green, fold over and collapse. At the point when leaves are attacked in the centre, they hang from the point of infection. On seed stalks, circular or prolonged lesions are produced and infected stalks break over from the heaviness of the seed umbel, and lead to seed shrinkage. The fungus additionally infects botanical parts and might be carried with the seed. In dry weather, the contagious growth vanishes and spots thin out, however when positive conditions prevail again the fungus resumes its growth (Gupta and Paul, 2001).

Under humid conditions infected leaves are covered with a light greyish purple mildew growth. The tissue under the growth becomes yellow and folds at the lesion and collapse. Infected bulbs are generally smaller compared to healthy bulbs. The disease causes leaves to turn light green or yellow, and curl downwards. A greyish-violet downy growth develops on infected leaf tissue. The disease is more common on seed crops than

bulb crops. Indications of pathogen can be seen on the surface of leaves or bloom stalk as violet growth of fungus, which later becomes pale greenish yellow and finally the leaves or seed stalks collapse (Schwartz and Mohan, 1995).

## 2.4 ETIOLOGY

*Peronospora destructor* (Berk.) Casp. ex Berk the causal agent of onion downy mildew is an obligate parasite in the family *Peronosporaceae* under the order *Peronosporales* of the class *Oomycetes* (Alexopoulos, 1962).

As different members of the class, it reproduces sexually by oval-shaped oospores measuring 15.8 - 43.2  $\mu$  which under favourable conditions germinate by germ tubes. Asexual reproduction is by lemon shaped sporangia measuring 53.4 - 61.7 x 24.2  $\mu$  (Jovicevic, 1964).

Sporangia are produced on dichotomously branched and determinate sporangiophores. These sporangia dependably germinate by germ tubes (Alexopoulos, 1962). *Peronospora destructor* parasitises onion tissues intercellularly deriving nutrients using haustoria which when mature curl and separates and measures 80 - 100 x 2.5 - 5 $\mu$  (Ikata and Yamauti, 1941).

This assimilation of nutrients can result into a green weight reduction of up to 53 per cent every night, and this green weight reduction has a positive correlation with the reduction in yield as revealed by Yarwood (1941). The expansive pyriform sporangia of *Peronospora destructor* are effectively distinguishable from the sporangia of other downy mildews, however the shape of the sporangia indicates considerable variety. The sporangial study done by Yarwood (1943) has demonstrated that at 22°C there is the development of fusiform sporangia, however at a lower temperatures they were predominantly pyriform. Sporangia framed in solid artificial light were sometimes constricted in the centre. The characters most helpful in distinguishing *Peronospora destructor* from closely related structures are the large pyriform sporangia, the filamentous haustoria, the dichotomous branching of the sporangiophores and the germination of the sporangia by germ tubes.

The downy mildews are believed to have arisen from *Phytophthora*-like ancestors and were shown to be embedded within this genus (Runge *et al.*, 2011; Thines, 2009). However phylogenomic investigations with a limited taxon sampling seem to favour an

independent evolution of *Phytophthora* and downy mildews (Matari and Blair, 2014; Seidl *et al.*, 2011).

*Peronospora* is a wide spread, primarily northern hemispheric genus, with diversity in families such as *Fabaceae*, *Caryophyllaceae*, *Ranunculaceae* and *Boraginaceae*, which in turn have a high diversity in Asia and the Mediterranean (Farr and Rosman, 2015).

## 2.5 PATHOGENICITY

Yarwood (1937, 1943) demonstrated that sporangia requires free water for germination and the germ tubes dependably entered the host through stomata. He additionally observed that inside the host, mycelia developed at the rate of 300  $\mu$ /hr. Sporangial germination is directly subjected to ideal humidity level.

The sporulating lesion produces sporangia for around 7 days continually under ideal conditions. This continuous supply of inoculum guarantees chances of infection. Pathogenicity related studies has demonstrated that sporangial germination on and inside onion leaf tissue was quicker than that in artificial media like rain water, water agar or distilled water (Cook, 1932). Katerfeld (1926) found that the incubation period extended from 10-15 days under the green house and 13-18 days in the field.

Cook (1932) from field observations, found that the development of the mildew is supported by abundant dampness and relatively lower temperatures. Under *in vitro* conditions, he found that the fungus sporulated over an extensive range of temperatures, free water on the leaves being a necessary condition and the incubation time frame was of 11-15 days. According to Viranyi (1974), an incubation period of 11-14 days and previous day exposure to light and minimum of 95 per cent RH were found as basic condition for sporulation of the fungus however the infection cycle took two humid evenings with the ideal temperature at 11 - 13°C.

Abd-Elrazik and Lorbeer (1980) had developed the procedure for the isolation and maintenance of *P. destructor* on onion plants in a greenhouse where leaves of onion plants were inoculated (leaves, either wiped or unwiped by cotton) in a wet chamber at 14°C for 24 hr in darkness. Sporulation can be induced by placing the infected plants overnight in a damp chamber at 14°C in darkness starting at 1600-1700 hours. Once isolated, the

pathogen was maintained by transferring 1 or 2 day old sporangia to healthy plants at an interval of 3 weeks.

The effects of temperature and relative humidity on *Peronospora destructor* sporulation on onion leaves were studied under controlled environmental conditions. Sporangia were produced most rapidly at 8 to 12°C after 5 hours of high humidity during dark periods. The greatest number of sporangia was produced at 100 per cent relative humidity (RH) and sporulation decreased to almost nil when humidity decreased to 93 per cent RH (Gilles *et al.*, 2004).

## **2.6 EPIDEMIOLOGY**

A close correlation was much of the time seen between the direction of the prevailing wind and the spread of the disease. The sporangial germination occurred most quickly (2-3 hours) at 10-18°C but took to some degree longer (4 hours) at 22°C and longest (5-7 hours) at 5°C. In field, two humid evenings were normally necessary for the occurrence of the whole infection cycle with incubation time of 11-14 days (Viranyi, 1974, 1975).

Downy mildew developed in a succession of steps of spread and intensification from a point source of inoculum in field plots. Appearance of epidemic was identified with a sequence of brief periods (< 1-2 days) required for sporulation and infection alternating with longer latent periods (10-16 days). Production of spores in the predawn hours required temperatures below 24° C on the earlier day and continuous high relative humidity (RH  $\geq$  95%) at favourable temperatures from 0200-0300 hours until first light (Hildebrand and Sutton, 1982). Accordingly the spore viability decreased exponentially with time in controlled condition and highest death rates recorded at warm temperatures (30°C) and high RH (95%).

In another field study, Leach (1985) found that the *Peronospora destructor* sporulates profusely at 100 per cent RH as long as the air is still however not when it is moving. According to him, the rate of spore germination was highest at 10°C but declines further with increase in temperature.

Germ tubes develop in liquid water and a continuous period of wetness has been required for infection to be completed. The temperature ideal for epidemics is 10-12°C, yet temperatures up to approx. 22°C can bolster fast disease development (Palti, 1989).

The capacity of sporangia to survive after discharge and during dissemination is a crucial factor in the study of disease epidemiology of downy mildew. Bashi and Aylor (1983) studied the germination of sporangia detached from the leaves under different conditions *in vitro* and revealed that germination of sporangia was up to 60 per cent at 76-95 per cent RH when kept at 10°C for 72 hours and 20 per cent at 33 per cent RH; though at 25°C, germination after 48 hours was roughly 20 per cent at 75 per cent RH and 0 per cent at 33 per cent RH.

Jespersion and Sutton (1987) developed downy mildew forecast based on quantitative and temporal relationship of temperature, rain, humidity, rate of dew deposition and dew period with infection cycle of pathogen. The pathogen requires presence of dampness in the form of free water from rain or dew on the leaf surface for the faster spread of the disease. For the initiation of infection, the pathogen requires cool temperature (< 22°C) and relative humidity more than 95 per cent. Gupta and Paul (2001) reported that cloudy days additionally support the development of disease because 8 hours of light is deadly to sporangia production.

Similarly downy mildew (*Peronospora parasitica*) in cauliflower often develops during the winter season. Cool damp weather with high relative humidity and air development invigorates disease development by promoting sporulation, spore dispersal and plant infection by the pathogen (Anonymous, 2005).

Successful infection by *Peronospora belbahrii*, the downy mildew of basil, is supported by cool to warm temperatures accompanied by high humidity. Profuse sporulation is facilitated by warm and wet conditions whereby the sporangiophores come out of the stomata, branch dichotomously and bear a single extensively ellipsoidal to subglobose sporangium at the tip (Mersha, 2012). *P. belbahrii* produces sporangia on sporangiophores under dark and humid conditions. Many studies suggest that sporulation of oomycetes occurs only during dark night hours (Cohen *et al.*, 2013).

The disease has a wide geographical conveyance, which can be credited to the versatility of the onion crop to many sorts of climate, including temperate, tropical and subtropical zones; the comparatively wide temperature extend under which conidia of the mildew can survive and the simplicity with which the pathogen can be spread by systemically infected bulbs. The effect of natural factors on sporulation by *P. destructor*

on onion plants are complex. Sporulation of *P. destructor* is a diurnal process, and the two times of light and obscurity are required, despite the fact that sporulation just occurs in haziness (Yarwood, 1937, 1943). High day temperature exceeding 24 to 25°C or exceeding 27, 28, 29 or 30°C for more than 8, 6, 4 or 2 hours respectively, were found to inhibit sporulation during subsequent nights. Around evening time, sporulation was found to occur just when humidity transcended 94 to 95 per cent RH and temperatures were in the vicinity of 4°C and 24°C.

## **2.7. DISEASE MANAGEMENT**

Onion production remains hampered because of number of diseases at all stages of development i.e. from nursery to its consumption as vegetable and it is assessed that production could be increased manifold if the crop is protected against different diseases.

Apart from reduction in crop yield, the disease additionally cause some harmful effects during harvesting, post harvesting, processing and marketing stage, which brings down the quality and export capability of the crop that significantly causes the economic loss.

The disease adjusts the cropping design and furthermore affect the local and export markets as well. Downy mildew management includes the methods such as planting time, tolerant or resistant cultivars, soil drainage, seed or bulb health stock, wind block (Schwartz and Mohan, 1995; Hoffmann *et al.*, 1996; Develash and Sugha, 1997 b; Krauthausen *et al.*, 2001).

The influence of variable fertilizer levels was likewise significant on downy mildew (Khan and Ahmed, 2001; Goncalves *et al.*, 2004).

### **2.7.1. FUNGICIDES**

To minimize the losses, use of resistant variety is the ideal way, but downy mildew is one of the worst diseases affecting almost every variety.

The principal methods for controlling the downy mildew is by applying fungicides also, the efficiency of protection relies upon their application time and disease development level. The current IPM recommendation is to apply fungicides at the first indication of downy mildew (Hoffmann *et al.*, 1996; Develash and Sugha, 1997 b; Wright *et al.*, 2002; Gianessi and Reigner, 2005). Generally, preventive fungicide sprays are

scheduled at a period of 7-10 days in order to control downy mildew and to guarantee higher yields and higher crop quality (Palti, 1989; De Visser, 1998).

On the other hand if, fungicide applications are undertaken during unfavourable weather conditions for controlling infection and disease spread then control measures are economically and ecologically superfluous. To achieve a superior control over epidemic of downy mildew in onion it is critical to make prophylactic sprayings as and when the main disease symptoms starts to show up i.e., when the disease incidence does not exceed 5.0 per cent. Fungicide application schedule should begin at about the time of the first sporulation-infection period (Jespersen and Sutton, 1987; Wright *et al.*, 2002).

Investigations conducted by Surviliené *et al.* (2008) during the period 2004-2007, considered the effect of various fungicides viz. Acrobat Plus (Dimetomorf 90 g kg<sup>-1</sup>, Mancozeb 600 g kg<sup>-1</sup>), Amistar 250 SC (Azoxystrobin 250 g l<sup>-1</sup>), Infinito SC 687.5 (Fluopicolide 62.5 g , Propamocarb HCL 625 g l<sup>-1</sup>), Penncozeb 75 DG (Mancozeb 750 g kg<sup>-1</sup>) and Signum 33 WG (Pyraclostrobin 67 g kg<sup>-1</sup>, Boscalid 267 g kg<sup>-1</sup>) on the epidemic advance of onion downy mildew. Three fungicidal spray applications significantly minimized the disease incidence in order of their superiority such as Infinito took after by Signum and Amistar; the biological efficacy of the fungicides averaged as 74.38-89.36 per cent and increase in the marketable onion yield was up by 4.3–26.3 per cent.

Many research workers have reported the control of this disease through the use of fungicides (Rakhimov and Sadygov, 1975; Loss and Stenina, 1975; Teviodale *et al.*, 1980; Georgy *et al.*, 1986; Smith *et al.*, 1986; Mir *et al.*, 1987; Palti, 1989; Golyshin *et al.*, 1991; Ahmad and Hakim, 2000; Hakim *et al.*, 2002).

An alternative control measure is the use of chemicals. A number of chemicals have been evaluated by different research workers to control the disease. Among these fungicides Metalaxyl and Cyomaxanil have proved the most effective in reducing the disease intensity from 72-88 per cent (Palti, 1989). Highly significant control of the disease was obtained with Ridomil MZ-71 WP (Tahir *et al.*, 1989).

Measures to reduce sources of infection and avert spread of disease are of extraordinary importance in controlling onion during mildew formation. Elimination of infected plants, hot water treatment of bulbs and eradication of diseased volunteer plants are recommended for the control of the disease. Nonetheless, currently the best method to

control downy mildew is the utilization of fungicides. These fungicides either inhibit germination, growth or multiplication of the pathogen (Agrios, 1995).

Mohibullah (1991) utilized a few fungicides (Antracol, Cuprisan 311-Super D, Dithane M-45, Nemispor, Penncozeb, Sandofan M, Ridomil MZ-71 and Tri Miltox Forte) against downy mildew of onion. Highly significant control of the disease was obtained with Ridomil MZ-71 WP and Sandofan M followed by Nemispor. Tahir *et al.* (1989) screened eight fungicides against downy mildew disease i.e. Antracol 70 WP, Liromanzeb 80 WP, Daconil 75 WP, Ridomil MZ-72 WP, Duter WP, Polyram Combi, Tri-Miltox Forte and Cupravit. Among these, Antracol 70 WP was the best, trailed by Ridomil MZ-72 WP. Efficacy of ten fungicides, viz Aliette, Antracol, Benlate, Cobox, Daconil, Derosal, Dithane, Polyram, Ridomil and Topsin-M against onion downy mildew and their contribution in increasing the yield was considered by Raziq *et al.* (2008). The results indicated that the application of fungicide Ridomil MZ at the rate of 2.5 g/l gave excellent control and is followed by Topsin-M and Aliette, each at the rate of 2.5g/l were comparatively more effective than alternate fungicides in reducing downy mildew severity and increasing yield by keeping 10 days interval for spraying.

Develash and Sugha (1997 a) conducted *in vitro* testing of thirteen fungicides (viz Metalaxyl + Mancozeb 72 WP, Oxadixyl + Copper oxychloride 50 WP, Oxadixyl + Mancozeb 64 WP, Benalaxyl + Mancozeb 73 WP, Folpet 80 WP, Mancozeb 75 WP, Zineb 75 WP, Benalaxyl + Copper oxychloride 37 WP, Copper oxychloride 50 WP, Captan 50 WP, Captafol 80 WP, Chlorothalonil 75 WP, Fosetyl-Al 80 WP) against *Peronospora destructor*. The results expressed that sporangia were most sensitive to combination of Metalaxyl + Mancozeb followed by Oxadixyl + Copper oxychloride and least to Fosetyl-Al.

### **2.7.2. SAR CHEMICALS**

Conventional techniques for disease administration have met with considerable success in managing plant diseases, but not true with downy mildew in particular. Utilization of fungicides has raised a larger scale concern for condition and wellbeing of human itself. Safe cultivars face the issue of resistance breakdown due to continuous use of systemic fungicides. Bio control is restricted due to lack of commercial formulations and has not been successful under field conditions due to loss of viability, poor

competitiveness, lack of proper delivery systems and environmental factors. Hence, there is a need to investigate new techniques based on activating the plant's own immune and defense barrier mechanism to control plant diseases (HC Sharma *et al.*, 2002).

Phenotypically, systemic resistance is shown as protection, which is dependable and active against wide spectrum of pathogens. The induced state is corroborated by an increase in production of a range of defense related products like pathogenesis related proteins (*Pr*-proteins), phytoalexins and signalling compounds (Heil and Bostock, 2002).

Induced systemic resistance is activated by various chemicals, non-pathogenic rhizobacteria, avirulent pathogens and pathogen derived elicitors. Exogenous application of chemicals like  $\beta$  aminobutyric acid (BABA), Acibenzolar-S-methyl (ASM), Isonicotinic acid (INA) and Salicylic acid (SA) have shown a multitude of defense response and can protect various crops like tomato, tobacco, pea, maize, cotton, rice, potato and other vegetables against viruses, fungi and bacteria by triggering a huge number of guard reaction (Kuc, 2001; Oostendorp *et al.*, 2001).

Few of these chemicals, such as BTH, have just been commercialized as plant guard activators (Bounaurio *et al.*, 2002; Agostini *et al.*, 2003; Ben-shalom *et al.*, 2003; Decapdiville *et al.*, 2003). Chitosan is a deacetylated type of N-acetylchito oligosaccharides containing poly D-glucosamine as its building blocks yet is structurally similar to cellulose. Chitosan is an exceptionally common polymer found in nature and has been accounted for in shells of crustaceans, insects and fungi (Hadwiger, 1999; Takechi *et al.*, 2000). Chitosan has been broadly researched to determine its capacity to elicit common barrier reaction and protect different crop plants from pathogenic parasites. Chitosan has been accounted for to protect tomato, cucumber, pea, melon, strawberry, lettuce against powdery mildews and other different diseases (Bhaskarareddy *et al.*, 1999). Elexa is a commercial formulation of 4 per cent chitosan utilized as a part of various vegetable and fruit crops like cucumber, pumpkin, zucchini, grapes and strawberries to control downy mildew, powdery mildew and grey mould diseases (Jones, 2000).

Exogenous application of SAR inducing compounds such as Acibenzolar-S-methyl (ASM), DL-3-aminobutyric acid (BABA), 2,6-dichloroisonicotinic acid (INA), Salicylic acid (SA) and Sodium salicylate (SS) is one approach to achieve induction of resistance against an extensive range of microbial pathogens in plants (Kessmann *et al.*, 1994; Sticher

*et al.*, 1997; Cohen, 2002; LaMondia, 2009; Walters *et al.*, 2005). Chitosan is very efficient in chelating some essential nutrients, metal ions and trace elements necessary for bacterial and fungal growth (El Hadrami *et al.*, 2010; Xing *et al.*, 2015) thus, inhibits thereby toxin production and microbial growth (Reddy *et al.*, 1997). Chitosan at low molecular weight acts as a potent biotic elicitor, able to induce plant defence responses and to activate different pathways that increase the crop resistance to diseases (Hadwiger, 2013; Katiyar *et al.*, 2014).

Mersha (2012) assessed five SAR inducers viz. Acibenzolar-S-methyl (ASM, Actigard 50WG), DL-3-aminobutyric acid (BABA), Isonicotinic acid (INA), Salicylic acid (SA) and Sodium salicylate (SS) under greenhouse for their efficacy to control basil downy mildew and discovered ASM and BABA as most promising SAR inducers when applied at ideal rate, by an efficient technique and at an appropriate timing. Application of phosphate to first leaves has prompted significant increase in activities of various enzymes such as phenylalanine ammonia lyases, peroxidase and lipooxygenase in second leaves and activities of these catalysts were increased further following pathogen challenge. Phosphates in a similar manner appeared efficacious to give disease control under field conditions. Along these lines,  $K_2HPO_4$  when sprayed over rice @ 50 mM reduced neck blast caused by the fungus *Pyricularia oryzae* by 29 and 42 per cent, with increases in grain yield in between 12 and 32 per cent (Mandahar *et al.*, 1998).

The activation of protective mechanisms in plant tissues with chitosan has inhibited the growth of taxonomically different pathogens (Vasyukova *et al.*, 2001). SAR chemicals has been considered as an alternative to chemical fungicides and also reported to influence the production of substances related to stress response such as phytoalexins and chitinases (O'Herlihy *et al.*, 2003). Kothari and Patel (2004) stated that in many plant pathogen interactions, R-Avr gene interactions results in hypersensitive response and at distal end of the plant, a broad spectrum resistance is induced known as Systemic Acquired Resistance (SAR). SAR is characterized by an accumulation of salicylic acid and pathogenesis-related proteins. Plant protection provided by induction of systemic resistance is an effective and simple approach of disease management.

Mechanism of resistance to different pathogens and pest (Conrath *et al.*, 2006) can be activated in different plant species by eliciting the systemic acquired resistance (SAR) or induced systemic resistance (ISR) pathways (Pieterse *et al.*, 2009). The primary

mechanism that are involved in resistance to *Plasmopara viticola* (Grape downy mildew) were described following treatment with BABA (Dubreuil-Maurizi *et al.*, 2010), sulphated laminarin (Trouvelot *et al.*, 2008), plant extracts (Godard *et al.*, 2009) and Fosetyl-Al (Dercks and Creasy, 1989). Experiment conducted by Akram and Anjum (2011), has proven that pathogen growth and development is restricted by structural and biochemical barriers in plant tissues under the influence of systemic resistance inducers.

Reuveni *et al.* (2000) also found that foliar sprays of phosphates were beneficial for cucumber by reducing infection with powdery mildew. The systemic protection by  $\text{KH}_2\text{PO}_4$  against powdery mildew in hydroponic culture was associated with 50 per cent increase in calcium content of cucumber leaves. Calcium ions play an important role in the production of salicylic acid and chitinase, both closely linked with SAR activity (Schneider-Müller *et al.*, 1994; Reuveni *et al.*, 2000).

## Chapter-3

# MATERIALS AND METHODS

---

### 3.1 SURVEY AND SURVEILLANCE

Systematic surveys were conducted during February-April, 2017-2018 at Research Farm of Seed Science and Technology, Pandah of Dr. Yashwant Singh Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh and Farmer's field for recording the prevalence of downy mildew in Solan district of Himachal Pradesh. The places included in the study were Pandah, Khaltoo, Nauni, Kandaghat, Oachghat, Dharampur and Maryog. In each of these places onion growing localities were surveyed by throwing a quadrat of one square metre in a field randomly at five places and number of healthy and diseased plants was counted and disease incidence was recorded.

The per cent disease incidence of downy mildew was worked out as per the following formula given by James (1974).

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

KEY SCALE	DESCRIPTION	INTENSITY (%)
1.	No symptom of disease.	0
2.	Only few leaves affected.	1
3.	Less than half of the plant affected.	5
4.	Most of the plants affected but the attack restricted mostly to one leaf per plant.	10
5.	All the plant affected and attack restricted to one or two leaves.	20
6.	Three to four leaves of almost every plant affected and the crops look fair green.	50
7.	Three to four leaves of almost every plant affected and the crops show blighted appearance.	75
8.	All the leaves of all plants severely attacked, greenness restricted to the central shoot only.	90
9.	Foliage completely blighted due to downy mildew, purple blotch and no green is seen at all.	100

For determining the severity of downy mildew, ten plants from each field were randomly selected and five such observations per field were recorded. Leaves were graded into different scales depending on per cent area infected as per Mohibullah (1991) by examining the lesions on leaves based on 0-9 scale.

The PDI (per cent disease index) was calculated by using the formula given by Mc Kinney (1923).

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total no. of ratings} \times \text{Maximum disease grade}} \times 100$$

### **3.2 IDENTIFICATION OF CAUSAL ORGANISM**

Infected leaves showing characteristics symptoms were collected and brought to the laboratory and thoroughly examined under microscope to detect the presence of mycelia, sporangia and sporangiophores. Morphological characters like Hyphal septation, branching of sporangiophores, size and shape of sporangia of downy mildew pathogen were recorded as suggested by Murphy and McKay (1926).

### **3.3 PATHOGENICITY TEST AND MAINTENANCE OF INOCULUM**

#### **3.3.1 Raising of nursery and transplantation of seedlings**

Seeds of the variety “Palam Lohit” were sown in nursery beds prepared from well-manured soil in the Research Farm of the Department of Seed Science and Technology of Dr. Yashwant Singh Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh. Forty five days after sowing, uniform and healthy seedlings were selected and transplanted in the pots of diameter 9 cm containing soil sterilized with formalin (4%) for conducting the pathogenicity test under artificially inoculated conditions and also for the rest of the experimentations.

#### **3.3.2 Preparation of inoculum for pathogenicity test and epidemiological studies**

Sporangia were collected from the surfaces of the infected leaves of plants raised under field with the camel's-hair brush and suspended in sterilized distilled water for utilisation in subsequent experiments. The suspension was filtered through muslin cloth.

The debris was held in the muslin cloth and the fungal spores in the distilled water were adjusted to a spore concentration of  $4 \times 10^4$  sporangia per ml with the help of haemocytometer.

### **3.3.3 Pathogenicity test**

#### **3.3.3.1 Detached leaf method**

Fresh healthy leaves of onion were brought to the laboratory of Plant Pathology and thoroughly washed with 0.1 per cent mercuric chloride for 15 seconds and then washed in distilled water thrice. These leaves were air dried within the folds of blotting paper and placed in plastic trays lined with thin layer of cotton and blotting paper completely saturated with distilled water, as per the leaf detached method suggested by Song *et al.* (1998). The leaves were inoculated by placing 0.50 ml sporangial suspension with the help of micropipette. After that incubated in BOD incubator for development of symptoms. Suitable control using water drop without sporangia, was also maintained. Incubation period (i) was worked out as mentioned below:

$$i = t_s - t_i$$

where, i = incubation period

$t_s$  = time of appearance of symptom

$t_i$  = time of inoculation of leaves

#### **3.3.3.2 Spray and drop inoculation method (under pot conditions)**

In order to prove the pathogenicity of the concerned pathogen, *Peronospora destructor* associated with downy mildew of onion by spray and drop inoculation method (Chen and Wang, 2005), forty five days old plants were taken. The surface of leaves to be inoculated was first surface sterilized with 0.1 per cent mercuric chloride for 15 seconds and then wiped with cotton pad soaked in distilled water (Abd-Elrazik and Lorbeer, 1980). Thereafter the sporangial suspension was sprayed and dropped (20  $\mu$ l) over the healthy leaves of onion plant. After the inoculation, the plants were kept under high humidity (> 95%) by covering the pots with moist perforated polythene bags and incubated in BOD incubator (14°C and 4 days) followed by 18°C for 8 days. Then pots containing inoculated plants were transferred to ambient environmental conditions. Suitable control (water drop

without sporangial suspension) was also maintained. Incubation period was calculated in a similar manner as mentioned above in 3.3.3.1.

### **3.3.4 Maintenance of inoculum**

In order to maintain the continuous supply of inoculum of pathogen during the research studies, the detached leaf method described in 3.3.3.2 was frequently used.

### **3.4 *In vitro* effect of different temperature (°C) regime on sporangial germination and germ tube formation**

In order to find out the optimum temperature for sporangial germination and germ tube formation of *Peronospora destructor*, sporangia were harvested in 50 ml of water by using camel's- hair brush from freshly sporulating leaves. The sporangial concentration was then adjusted to  $4 \times 10^4$  sporangia per ml with the help of haemocytometer. A drop (10  $\mu$ l) of sporangial suspension was placed in each cavity of cavity slides and placed on triangular glass rod and finally kept in Petriplates (90 mm) containing thin layer of cotton wool on both surfaces moistened with sterilized distilled water. Then Petriplates were incubated in BOD incubator at 5°C, 10°C, 15°C, 20°C, 25°C and 30°C. Three replications were maintained for each temperature and design followed was Completely randomised design (CRD). Germination of sporangia were recorded at an intervals of 12 hours, 24 hours and 36 hours by counting the empty sporangia and per cent germination was calculated by using the following formula:

$$\text{Spore germination (\%)} = \frac{\text{Number of empty sporangia}}{\text{Total number of sporangia}} \times 100$$

After 12, 24, 36 hours of incubation, spores were also examined for measuring germ tube length using micrometry under microscope by calibrating ocular and stage micrometers.

### **3.5 *In vitro* effect of different relative humidity (%) levels on sporangial germination and germ tube length formation**

In order to find out the effect of various relative humidity levels (%) at the best temperature obtained in the aforementioned experiment on sporangial germination and germ tube formation of *Peronospora destructor*, sporangia were harvested and made into a

solution of required concentration in a similar way as the experiment explained in temperature studies (3.4). After that Petriplates containing cavity slides were then incubated at 15°C at different humidity levels viz. 0, 56.8, 75.6, 82.9, 95.1 98.5 and 100 per cent by using the method given by Stevens (1916). For this aqueous solution of sulphuric acid, having specific gravity of 1.8 was used to maintain constant humidity. Since sulphuric acid solution is hygroscopic in nature and does not affect the growth of fungus. Germination of sporangia were recorded at 12 hour, 24 hour and 36 hours by counting the empty sporangia and calculating germination per cent by using the following formula as discussed in 3.4.

The germ tube length was also calculated by using micrometry at different time intervals of incubation, under microscope by using calibration factor attained by ocular and stage micrometers. The design of the experiment followed was CRD with three replications in each treatment.

### **3.6 Factors affecting the progression of disease under natural epiphytotic conditions**

Disease infection is affected by prevalence of different meteorological factors which are mainly temperature, relative humidity and rainfall. Therefore in order to study their effect on the progress of downy mildew of onion under natural epiphytotic conditions, data on disease severity were recorded at 4 days interval by examining 30 plants in total with the first appearance of disease under field conditions with effect from the last week of February 2018 to the second week of May, 2018. The per cent disease index (PDI) was calculated by using McKinney's formula (1923) as described earlier (3.1). The data on weather parameters were collected from the Meteorological Observatory, Department of Environmental Science, College of Forestry, Dr. Yashwant Singh Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh to find out their impact on disease initiation and subsequent spread. Correlation and regression coefficients were also calculated by following the procedures given by Gomez and Gomez (1984) and regression lines were developed. The time of appearance of disease in days and disease severity at each interval was also calculated.

### **3.7 Disease Management**

Evaluation of fungicides under *in vitro* conditions was carried out to find out the most effective fungicide against the test pathogen (*P destructor*), 4 non-systemic

fungicides and 3 combi-products were evaluated under *in vitro* conditions by spore germination method (Zarcovitis, 1965) at different concentrations. The fungicides evaluated during the course of present investigation were as under

<b>Non Systemic Fungicides</b>	<b>Concentrations (ppm)</b>
Dithane M-45 (Mancozeb 75% WP)	500, 750, 1000
Blitox 50 (Copper oxychloride 50% WP)	500, 750, 1000
Kavach (Chlorothalonil 75% WP)	500, 750, 1000
Antracol (Propineb 70% WP)	500, 750, 1000
<b>Combi-products</b>	<b>Concentrations (ppm)</b>
Ridomil Gold 68 WG (Mancozeb 64% + Metalaxyl 4%)	200,300,500
Nativo 75 WG (Tebuconazole 50%+ Trifloxystrobin 25%)	200,300,500
Cabrio Top 60 WG (Pyraclostrobin 5% + Metiram 55%)	200,300,500

### 3.7.1 Spore germination inhibition method

Different concentrations of each fungicide were prepared in the sterilized distilled water. Simultaneously the spore suspension ( $4 \times 10^4$  sporangia/ml) was also prepared from freshly harvested sporangia by using camel's-hair brush. One drop (10  $\mu$ l) of each double strength fungicide solution was dropped in the cavity slides followed by one drop (10  $\mu$ l) of sporangial suspension. The cavity slides were then placed on a glass rod kept in a Petriplate containing sterilized distilled water at the bottom and sterilized moistened cotton wool lining the lower and inner surface of upper lid. The Petriplates containing cavity slides were finally kept in BOD incubator at 15 °C with 95% relative humidity. The experiment was laid out in a Completely Randomised Design (CRD) and each treatment was replicated three times. The readings on germination of sporangia and sporangial germ tube length were recorded after 12, 24 and 36 hours by placing cavity slides under compound microscope and per cent inhibition in germination of each fungicides was calculated by adopting the formula given by Vincent,1947 as below:

Per cent spore inhibition (I) =  $C-T/C \times 100$  where, C= Spore count in control (numbers), T= spore count in treatment (numbers)

### 3.7.2 Evaluation of fungicides and SAR chemicals under *in vivo* conditions

Five most effective fungicides obtained from non systemic as well as Combi-products viz. Dithane M-45@ 0.25% (Mancozeb 75WP), Antracol @ 0.3% (Propineb

70% WP), Ridomil Gold 68 WG (Mancozeb 64% + Metalaxyl 4%) @ 0.25%, Nativo 75 WG (Tebuconazole 50%+ Trifloxystrobin 25%) @ 0.03% , Cabrio Top 60 WG (Pyraclostrobin 5% + Metiram 55%) @ 0.03% along with systemic acquired resistance (SAR) chemicals like Chitosan (5ppm), Potassium orthophosphate (20ppm) and BABA (10ppm) individually were evaluated for their effect on per cent disease severity of downy mildew under field conditions. The experiment was laid out in Randomised Block Design (RBD). Each treatment was replicated thrice by keeping the control. The experiment was conducted in Pandah Research Farm of Department of Seed Science and Technology of Dr. Yashwant Singh Parmar University of Horticulture & Forestry, Nauni, Solan, HP. Plants of cultivars “Palam Lohit” after 45 days of sowing in nursery were transplanted in the main field with spacing of 10×5 cm. All standard agronomical practices were carried out at specified time. SAR chemicals were applied once at 3-4 leaf stage of the plant initially (without disease) and later the same plants were sprayed two times at 15 days interval, after the appearance of the disease. Whereas fungicides were sprayed immediately at their recommended concentration after the onset of the first disease symptoms under the field conditions with a total of 3 sprays applied at fortnight intervals. The observations were recorded on per cent disease severity at 7 days interval, plant height (cm) was taken at 30 days interval and bulb yield (kg/plot or t/ha) at the maturity of the crop i.e. at the time of harvesting.

### **3.8 Statistical Analysis**

Data recorded in different experiments conducted above is subjected to statistical analysis wherever necessary using standard procedures as per Gomez and Gomez (1984) and Panse and Sukhatme (2000). The significance of difference was tested at 1% and 5% level of probability.

## Chapter-4

# RESULTS AND DISCUSSION

---

Results of the present investigation entitled “**Studies on Downy mildew of Onion (*Allium cepa* L.)**” are described under following headings

### 4.1 DISEASE SURVEY

### 4.2 SYMPTOMATOLOGY

### 4.3 IDENTIFICATION OF CAUSAL ORGANISM

### 4.4 PATHOGENICITY TEST

### 4.5 MAINTENANCE OF INOCULUM

### 4.6 EPIDEMIOLOGICAL STUDIES

### 4.7 DISEASE MANAGEMENT

### 4.1 DISEASE SURVEY

To assess the prevalence and magnitude of downy mildew of onion, systematic surveys of onion growing areas located in Solan district of Himachal Pradesh were conducted during the crop season 2017 – 2018. The data recorded on downy mildew severity is presented in Table 4.1 which reveals that the disease was prevalent as very low, moderate and severe form in onion growing areas of Solan of Himachal Pradesh surveyed during course of present investigations.

**Table 4.1 Severity of onion downy mildew disease at different locations in Solan of Himachal Pradesh during 2017-18**

Location (s)	Disease incidence(%)	Disease index (%)
Nauni	30.33	21.76
Pandah	39.24	58.65
Khaltoo	31.25	35.86
Maryog	22.3	28.43
Dharampur	10.24	4.78
Kandaghat	14.3	6.76
Oachghat	18.33	21.16
<b>Mean</b>	<b>23.71</b>	<b>28.96</b>

From the data (Table 4.1), it is also evident that the disease was widespread in all the seven areas surveyed. However, disease incidence was highest (39.24 %) in Pandah and lowest (10.24 %) in Dharampur. The per cent disease index (PDI) of downy mildew ranged from 4.78 to 58.65 per cent. Highest PDI was recorded at Pandah (58.65 %) followed by Khaltoo (35.46%), Maryog (28.43%) and Nauri (21.76%) in contrast to lowest PDI at Dharampur (4.78%). The overall data revealed that both the incidence and the PDI of the disease were higher in Pandah followed by Khaltoo, especially in the areas where dew prevailed for longer period in winter season. The intensity of the disease was reported to be quite high up to 100 per cent in different parts of world (Kennedy, 1998). It has got global occurrence and becomes more relevant in temperate regions (Domingues and Tofoli, 2009). The distribution of this pathogen is global (Agrios, 1995; Brewster, 2001) and affects crop yields by more than 60% (Surviliené *et al.*, 2008).

## **4.2 SYMPTOMATOLOGY**

During the course of survey the disease was found to appear at all stages of growth. The characteristic symptoms that were commonly observed are described below:

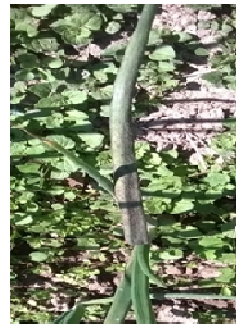
### **4.2.1 On leaves**

Disease is characterized by circular or elliptical lesion (as if caused by mechanical injury) that become white slightly sunken and roughened (Plate. 1a.). Such lesions were found on the single side of the stalk and mostly on the older leaves. With the passage of time these lesions were covered with the grey downy masses that turned purplish after heavy rain (Plate. 1b.) After the infection leaf colour changes from light green to yellow followed by necrosis of particular area (Plate. 1c.). Downy growth associated with infected tissues usually consist of sporangia and the sporangiophores having characteristic branching. Infection at younger stage has caused systemic infection by inducing stunting, defoliation, declining and death of plant tissues.

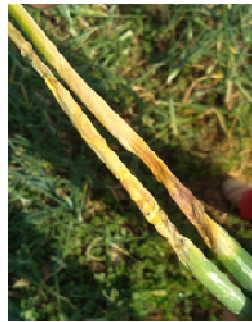
Affected leaves were light green and showed tip die-back in the form of necrosis and curling (Plate. 1d.). Pattern of yellowing in the field was seen extended out in the direction of prevailing wind. In case of severe downy mildew infestation lesions become necrotic followed by girdling and collapsing of that portion of leaf that leads to hanging of leaf stalk from the point of necrosis (Plate. 1e.). Similar kinds of symptoms were reported by many workers (Schwartz and Mohan, 1995; González *et al.*, 2010; Maude, 1990; Sharma *et al.*, 2002; Van Doorn 1959).



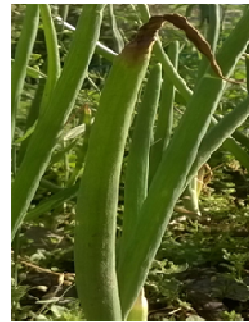
**Plate 1a Circular and elliptical lesion**



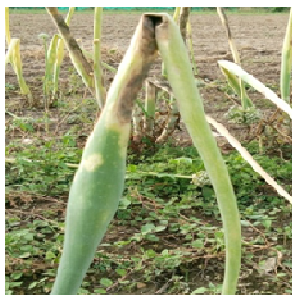
**Plate 1 b Grey downy cover**



**Plate 1c Yellowing of leaves**



**Plate 1d Tip die-back**



**Plate 1e Hanging down of leaf stalk**



**Plate 1f Bottle-necked bulbs**



**Plate 1g Small sized infected bulb as compared to healthy bulb**

**Plate 1 Symptomatology of downy mildew of onion**



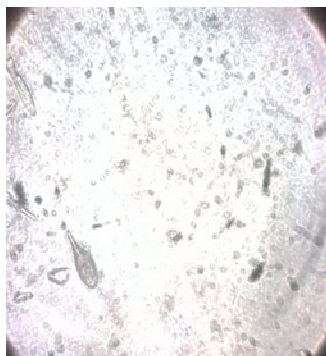
**Plate 2a Aseptate and hyaline mycellium**



**Plate 2b Sporangiphore**



**Plate 2c Sporangia**



**Plate 2d Germ tube production**

**Plate 2 Morphological identification of downy mildew pathogen**

#### 4.2.2 On bulbs

Few bottleneck bulbs were also seen due to severe infection of aerial portions of the plant (Plate. 1f.). Downy mildew fungus has also affected the bulb development and reduced yield, as infected bulbs were smaller than healthy bulbs (Plate. 1g.). Scholten *et al.*, 2007 and Ransomanski (1967) has observed similar kind of symptoms on bulbs that cause reduced quality and yield.

#### 4.3 IDENTIFICATION OF CAUSAL ORGANISM

Identification of causal organism of downy mildew fungus was done on the basis of morphological characters which were compared with the identification key given by Murphy and McKay (1926) by bringing the infected leaves from various locations.

**Table 4.2 Morphological characters of causal organism (*Peronospora destructor* (Berk.) Casp. ex Berk.) of downy mildew of onion**

STRUCTURE	SHAPE	SIZE (µm)
Mycelia	Hyaline, Aseptate	3.7-6.8 (width)
Sporangiophore	Dichotomously branched	Variable size
Sporangia	Pyriiform/ Fusiform	50.4-65.8 × 24.6-25.2

The microscopic examination of the fungus revealed that the mycelium was aseptate hyaline and dichotomously branched measuring 3.6 to 6.9 µm in width, (Plate. 2a.) whereas size of sporangiophores varies greatly (Plate. 2b.). The size of sporangia ranged from 50.4 to 65.8 × 24.6-25.2 µm and colour of sporangia was hyaline, having pyriform to fusiform shape (Plate. 2c.). On the basis of explained morphological characters the pathogen was identified as *Peronospora destructor* (Berk.) Casp. ex Berk. Above findings were well supported by the work of Langston and Sumner (2000) and Göker *et al.* (2007).

#### 4.4 PATHOGENICITY TEST

Pathogenicity test to confirm the pathogen *Peronospora destructor*, was conducted on 25 days old plants of cultivars “Palam Lohit” by spraying sporangial suspension of  $4 \times 10^4$  sporangia / ml and Koch’s postulates were proved. Symptoms on leaves were noticed after 12 days of inoculation under pot conditions by using Spray and drop inoculation method (Plate. 3.) The leaves that were wiped with cotton developed symptoms two days earlier than those not wiped. While in detached leaf method, the leaves inoculated with sporangial suspension

supported the growth of sporangia, 8 days after inoculation (Plate. 4.). Thus detached leaf method also yielded fast and maximum (60 %) of sporangia at higher humidity (95-96 %) at 10-15 °C for 72 hours but decreased as the relative humidity levels dropped down.

**Table 4.3 Pathogenicity of *P. destructor* causing downy mildew of onion**

METHOD	INCUBATION PERIOD (DAYS)
Detached leaf method	8
Spray and drop	
• Pre-inoculation wiping (Cotton pad)	12
• No pre-inoculation wiping	14

Pre inoculation wiping of the leaves has shown early symptoms as compared to the unwiped surface as wiping has increased the wettability and removed the surface wax that has supported better penetration and germination of sporangia (Abd-Elrazik and Lorbeer, 1980). Such observation has also been reported by Develash and Sugha (1997a). Latency period up to the development of new sporulation lasts from 9 to 16 days (Yarwood, 1943; Van Doorn, 1959; Maude, 1990; Schwartz and Mohan, 1995).

#### 4.5 MAINTENANCE OF INOCULUM

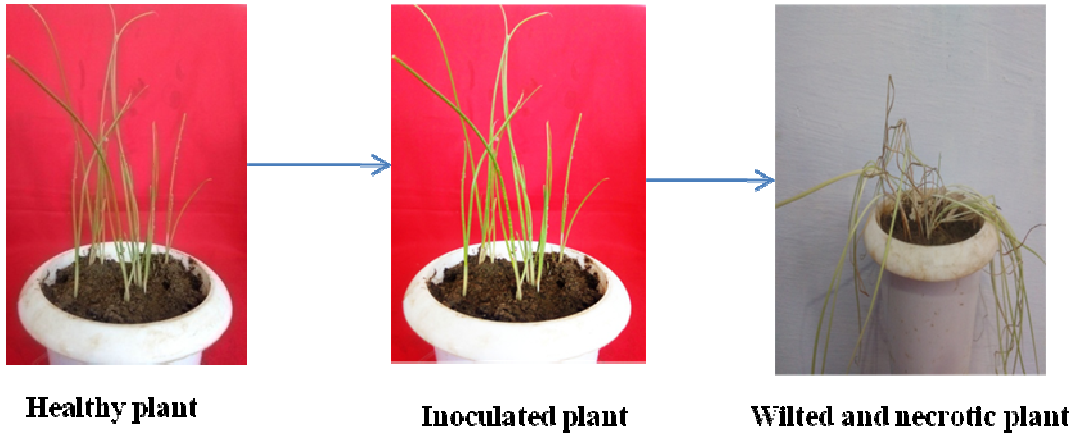
The inoculum was maintained by following Detached leaf method for continuous supply of inoculums for different experimentation. Sporulation on detached leaves was obtained in 8 days and after that pathogen was maintained by transferring two days old sporangia to healthy leaves.

#### 4.6 EPIDEMIOLOGICAL STUDIES

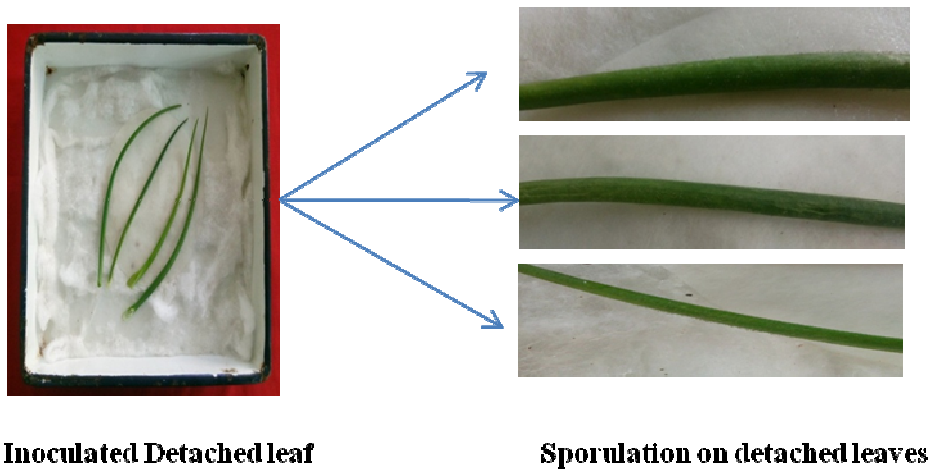
##### 4.6.1 *In vitro* effect of different temperature regimes on sporangial germination and germ tube length production

The data pertaining to effect of temperature on sporangial germination and germ tube length of *Peronospora destructor* causing onion downy mildew recorded and presented in Table 4.4. Different temperature levels viz; 5, 10, 15, 20, 25 and 30 °C were determined for knowing the sporangial germination and germ tube length production. No sporangia germinated at 5, 25 and 30 °C however sporangia germination and germ tube length production initiated at limited temperature regimes of 5, 10 and 20 °C after 12 hours of incubation. Maximum spores (73.96%) germinated at 15°C temperature at 36 hours giving highest germ tube length (202.98µm). Overall trend showed an increase in spore germination and germ tube length production from 10 to 15 °C, maximum (68.74%, 194.63 µm) being at

## **PATHOGENECITY TEST**



### **Plate 3 Spray and drop inoculation method**



### **Plate 4 Detached leaf method**

15°C and thereafter a sharp decline at 20 °C was observed in both the cases giving minimum (7.91%) germination and germ tube length (64.06 µm) of the sporangia, respectively. Time period studied after 24 hours has not played much role in enhancing the spore germination and germ tube length elongation as the 24 hours and 36 hours have a little increase in individual interactions and were found statistically at par when overall impact is observed. Thus, results of the present study concluded that the pathogen preferred 15 °C temperature after 24 h of incubation as best for spore germination and formation of germ tube and did not requires the temperature regimes of 5, 25 and 30 °C as no response towards spore germination was noticed.

Similar findings with respect to sporangial germination of downy mildew infection on various crops were reported by other workers (Bashi and Aylor, 1983; Kofoet, 1986; Hilderbrand and Sutton, 1984; Van Doorn, 1959; Yarwood, 1943). Armstrong and Sumner (1935) using incubation periods of 22 and 48 hr reported the optimum germination range of *Peronospora tabacina* to be 15-23°C. No germination was reported at temperatures higher than 29°C. According to Palti (1989) the rate of spore germination is highest at 10 °C and declines with the rise in temperature which is contrary to our study, as 15 °C has supported maximum germination of sporangia. The time taken for sporulation by *P. belbahrii* is completed within about 11 hours from the onset of darkness in moisture saturated atmosphere at 18°C. Similar results were also obtained with several other *Peronosporales*, including *Sclerospora sorghi* in maize (Cohen and Sherman, 1977), *Pseudoperonospora cubensis* on cucumber (Cohen and Eyal, 1977), *Peronospora tabacina* on tobacco (Cohen, 1977), *Phytophthora infestans* on potato (Cohen *et al.*, 1975) and *Plasmopara viticola* on grapes (Rumbolz *et al.*, 2002).

#### **4.6.2 *In vitro* effect of different relative humidity (%) levels on sporangial germination and germ tube length production**

The data obtained on effect of eight (100, 98.5, 95.1, 88.5, 82.9, 75.6, 56.8 and 0%) relative humidity (RH) levels on germination and germ tube length of sporangia are given in Table 4.5. The results revealed that out of various levels of relative humidity, sporangia in deionised water germinated only at 95.1, 98.5 and 100 per cent relative humidity by giving germination percentage of 33.12, 62.35 and 71.40 per cent in increasing order according to the increase in the RH levels. The levels of relative humidity also showed the concurrence in germ tube length formation which was lowest (78.23 µm) at 95.1 per cent RH and highest at 100 per cent RH (126.91 µm) with a gradual increase in germ tube length as the levels of RH increased.

**Table 4.4 Effect of different temperature regimes on sporangial germination and germ tube length of *Peronospora destructor***

Temperature (°C)	Sporangial germination (%)			Mean	Germ tube length (µm)			Mean
	12 hours	24 hours	36 hours		12 hours	24 hours	36 hours	
5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<b>0.00</b> <b>(0.00)</b>	0.00	0.00	0.00	0.00
10	34.92 (36.20)	44.93 (42.07)	45.03 (42.79)	<b>41.59</b> <b>(40.11)</b>	99.76	115.25	116.95	110.75
15	60.89 (42.07)	72.66 (58.46)	73.96 (58.97)	<b>68.74</b> <b>(56.06)</b>	180.39	201.74	202.98	194.62
20	6.03 (14.19)	8.85 (17.29)	9.96 (17.96)	<b>7.91</b> <b>(16.26)</b>	55.36	68.41	69.51	64.06
25	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<b>0.00</b> <b>(0.00)</b>	0.00	0.00	0.00	0.00
30	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<b>0.00</b> <b>(0.00)</b>	0.00	0.00	0.00	0.00
Mean	<b>16.98</b> <b>(16.95)</b>	<b>21.07</b> <b>(19.64)</b>	<b>21.97</b> <b>(20.04)</b>		<b>55.92</b>	<b>64.4</b>	<b>65.94</b>	
CD <sub>0.05</sub>	Temperature (T)=0.6 Time (H)= 0.4 TxH=01.0				T= 1.02 H= 0.72 TxH=1.8			

\* Figures in the parentheses are arc sine transformed values

**Table 4.5 Effect of different relative humidity levels on sporangial germination and germ tube length of *Peronospora destructor***

Relative humidity (%)	Sporangial germination (%)			Mean	Germ tube length (µm)			Mean
	12 hours	24 hours	36 hours		12 hours	24 hours	36 hours	
<b>100</b>	64.21 (53.24)	75 (59.1)	75.9 (59.78)	<b>71.40</b> <b>(57.73)</b>	100.06	140.33	142.63	<b>126.91</b>
<b>98.5</b>	55.91 (48.38)	65.57 (54.05)	66.24 (54.65)	<b>62.35</b> <b>(52.16)</b>	77.87	120.11	122.11	<b>106.03</b>
<b>95.1</b>	24.3 (29.52)	37.53 (37.76)	38.53 (37.96)	<b>33.12</b> <b>(35.02)</b>	63.33	85.68	86.68	<b>78.23</b>
<b>88.5</b>	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<b>0.00</b> <b>(0.00)</b>	0.00	0.00	0.00	<b>0.00</b>
<b>82.9</b>	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<b>0.00</b> <b>(0.00)</b>	0.00	0.00	0.00	<b>0.00</b>
<b>75.6</b>	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<b>0.00</b> <b>(0.00)</b>	0.00	0.00	0.00	<b>0.00</b>
<b>56.8</b>	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<b>0.00</b> <b>(0.00)</b>	0.00	0.00	0.00	<b>0.00</b>
<b>0</b>	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	<b>0.00</b> <b>(0.00)</b>	0.00	0.00	0.00	<b>0.00</b>
<b>Mean</b>	<b>18.05</b> <b>(15.74)</b>	<b>22.26</b> <b>(18.96)</b>	<b>22.58</b> <b>(19.01)</b>		<b>30.16</b>	<b>43.27</b>	<b>45.65</b>	
<b>CD<sub>0.05</sub></b>	RH (R)=0.39 Time (H)= 0.24 RxH= 0.68				<b>CD<sub>0.05</sub></b>	RH (R)= 2.18 Time (H )=1.34 RxH= 3.78		

\* Figures in the parentheses are arc sine transformed values

In interaction studies, highest sporangial germination was recorded up to 75.9 per cent after 36 hours of incubation at best 15 °C reported from the previous findings followed by 24 hours at 100 per cent RH level and lowest (24.30 %) sporangial germination at 95.1 per cent RH after 12 hours. Similar levels resulted into maximum germ tube length (142.63µm) at 36 hours and lowest (63.33µm) after 12 hours. In general there was a significant reduction in sporangial germination (33.12%) and germ tube length (78.23 µm) at 95.1 per cent RH. However, no sporangia germinated below the levels i.e. 88.5 to 0 per cent RH. Hence very high relative humidity above 95.1 per cent to 100 per cent is needed for the sporangial germination at optimum temperature of 15°C. Van Doorn (1959) and Leach (1985) has also reported similar results showing that lower relative humidity causes rapid loss of viability of sporangia. Cohen *et al.* (2013) has also reported that *Peronospora belbahrii* requires moisture-saturated atmosphere in the dark for sporulation. Severe mildew outbreaks under semi-arid and rainless conditions is attributed due to favourable early morning temperatures and the limited hours of high humidity (Palti, 1989). Sporangial germination was decreased to almost zero level due to decrease in the relative humidity of 93 per cent and hence no formation of germ tube as reported by Gilles *et al.* (2004).

#### **4.6.3 Role of meteorological factors on disease epiphytotics under field conditions**

In order to study the effect of meteorological factors on the progress of disease under field conditions, experiment was conducted at Pandah Research Farm of Department of Seed Science and Technology of Dr. Yashwant Singh Parmar University of Horticulture & Forestry, Nauni, Solan, HP under natural epiphytotic conditions during the cropping season, 2017-2018 The data on severity of the disease was recorded after the establishment of the crop and once the disease appeared in the field, observations were taken at four days intervals commencing from 25<sup>th</sup> Feb to 15<sup>th</sup> May, 2018 and are presented in Table 4.6

The data on weather parameters and disease development revealed that the disease did not appear during cropping period, 2017 and till mid of February, 2018 but only in the 4<sup>th</sup> week (25–28 February) the disease initiated with the prevalence of congenial weather conditions such as relatively high average relative humidity of 52.8 per cent accompanied by cumulative rainfall (3.4 mm), mean maximum (22.12 °C) and minimum (7.6 °C) temperatures.

There was increasing trend in disease level and it assumed serious proportion (70 to 72.76%) during the mid April, 2018 however maximum 72.76 per cent disease index was

noticed on first week, 6 - 9 April of observation dates when the mean maximum, minimum temperatures and average relative humidity remained within the range of 25.3 to 27.5 °C, 5.8 to 12.3 °C and 58 to 68 per cent that leads to prolific sporulation of *Peronospora destructor*. The disease however decreased slowly thereafter and reached to 66.65% up to the harvesting time of the crop due to the decomposed lower leaves and emergence of the new leaves. These results to a greater extent also corroborate the findings of various workers. (Hiura, 1930; Cook, 1932; McKay, 1939; Hildebrand and Sutton, 1982; Nakov, 1972; Sangeetha and Siddaramaiah, 2007; Randomanski, 1967; Viranyi, 1974; Premila and Sophiarani, 2015; Premila, 2013). Viranyi (1975) observed that germination occurred most rapidly (2-3 h) at 10-18°C and took somewhat longer (4 h) at 22°C and longest (5-7 h) at 5°C . Temperatures of 10-12°C are apparently optimal for most stages, 14-18 °C is still quite favorable, but temperatures above 22 °C, rapidly become unfavorable, especially at lower humidities.

**Table 4.6 Effect of meteorological factors on the development of downy mildew on onion during 2017-18**

Date of Observation	Mean Maximum temperature (°C)*	Mean Minimum temperature (°C)*	Cumulative rainfall (mm)*	Average relative Humidity (%)*	Disease index (%)
25 Feb-28 Feb	22.12	7.6	3.4	52.8	3.55
01 Mar-04 Mar	21.45	8.2	1	55	8.67
05 Mar - 08 Mar	22.5	5.6	3	47	16.99
09 Mar-12 Mar	24.35	7.8	0	38	23.43
13 Mar-16 Mar	24.2	6.4	0	45.75	33.56
17 Mar-20 Mar	24.45	9.8	0	40	48.67
21 Mar-24 Mar	23.27	8	3.35	66.12	51.34
25 Mar - 28 Mar	26.5	8.2	0	51	53.66
29 Mar-1 Apr	29.3	7.4	0	67	58.11
02 Apr-05 Apr	28.5	9.4	0	68	58.78
06 Apr-09 Apr	25.3	6.7	0	68	72.76
10 Apr-13Apr	26.4	5.8	0	50	71.11
14 Apr-17Apr	26.2	12.3	0	44	70.56
18 Apr-21Apr	27.5	10.8	0	58	70.09
22 Apr-25Apr	24.3	13.2	0	59	69.56
26Apr-29Apr	25.4	16.5	1	67.5	68.22
30 Apr-3 May	23.2	18.2	3.5	65	68.84
4 May-7May	24.5	16.3	1.5	51	67.21
08 May-11May	25.2	15.7	2	53	67.02
12 May-15 May	26.6	17.23	2.2	48	66.65

\*Average of four days

## 4.6.2 Correlation and regression analysis

Simple, partial and multiple correlations were worked out between per cent disease index (PDI) and meteorological factors (mean maximum temperature, mean minimum temperature, cumulative rainfall and average relative humidity) for the cropping season 2017-18.

### 4.6.2.1 Simple correlation

The data presented in Table 4.7 indicates the role of mean temperature (maximum, minimum) and average relative humidity and found them positively correlated with the disease development but the cumulative rainfall did not showed any impact and hence was negatively correlated with the progress of the disease. Simple correlation coefficient between the onion downy mildew and mean temperature maximum (0.774) was positive and highly significant. Similarly mean temperature minimum (0.620) and average relative humidity (0.353) were positive and highly significant, significant, respectively while cumulative rainfall (-0.178) developed negative correlation and did not found significant. Gupta and Paul (2001) concluded that the downy mildew pathogen require cool temperature (22°C) and relative humidity greater than 97 per cent with cloudy days additionally supporting the development of the disease and found them positively correlated.

**Table 4.7 Simple correlation coefficients between per cent disease index (PDI) and meteorological factors**

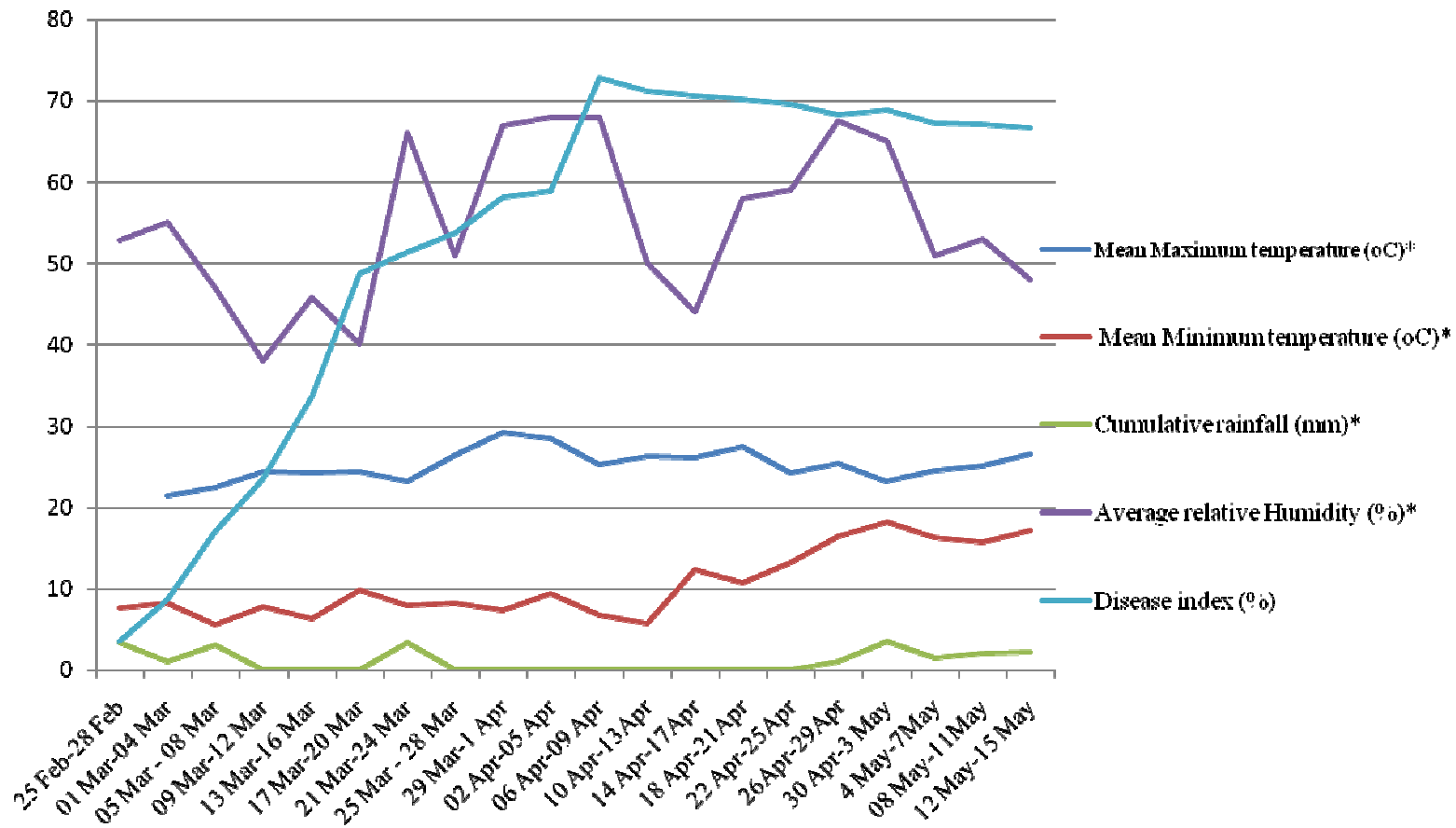
Meteorological factors	Year 2018
Maximum temperature (°C) x PDI	0.774**
Minimum temperature (°C) x PDI	0.620 *
Cumulative rainfall (mm) x PDI	-0.178
Average Relative humidity (%) x PDI	0.353

\*\* - significant at 1 % level

\* - significant at 5 % level

### 4.6.2.2 Partial correlation

Partial correlation coefficients were worked out using all the combinations of PDI with mean maximum temperature, mean minimum temperature, cumulative rainfall and average relative humidity and are presented in Table 4.8. It is evident from the results that the partial correlation between downy mildew index and mean maximum temperature (0.586) was highly significant and mean minimum temperature (0.547) was significant and positive.



**Figure 1: Graphical representation of different meteorological parameters on downy mildew of onion (cv. Palam Lohit) under field conditions**

Whereas a negative and non-significant partial correlation coefficient analysis observed with cumulative rainfall (-0.132) however with respect to relative humidity a partial correlation coefficient (0.238) indicated positive impact of this factor but found non-significant.

**Table 4.8 Partial correlation coefficients between per cent disease index (PDI) and meteorological factors**

Meteorological factors	2018
Maximum temperature (°C) x PDI	0.586 **
Minimum temperature (°C) x PDI	0.547
Rainfall (mm) x PDI	-0.132
Relative humidity (%) x PDI	0.238

\*\* - significant at 1 % level

\* - significant at 5 % level

#### 4.6.2.2 Multiple regressions

The multiple coefficient of determination ( $R^2$ ) was calculated to measure the contribution of linear function of independent variables, such as mean maximum temperature ( $T_{max}$ ), mean minimum temperature ( $T_{min}$ ), cumulative rainfall (Rf) and average relative humidity (RH) on dependent variable i.e. PDI and is presented in Table 4.9 given below:

**Table 4.9 Multiple regression equation and coefficient of determination of disease development based on meteorological factors**

Year	Regression equation	$R^2$
2018	$Y = -125.687 + 5.39X_1^{**} + 2.17X_2^* - 1.51X_3 + 0.33X_4$	0.874

\*\* - significant at 1 % level

\* - significant at 5 % level

Where, Y = Disease index (%);  $X_1$  = Maximum temperature (°C);  $X_2$  = Minimum temperature (°C);  $X_3$  = Rainfall (mm) and  $X_4$  = Average relative humidity (%)

Multiple correlation coefficients, as depicted in Table 4.9 between the onion downy mildew (PDI) and other meteorological factors indicated that 87.40 per cent disease index was caused by mean maximum temperature, mean minimum temperature, average relative humidity and cumulative rainfall collectively. While the rest of the variations might have contributed the change or variation due to unexplained variables (error variation) and or the factors not included in the present investigations.

The data on weather parameters and disease development revealed that the disease appeared in the 4<sup>th</sup> week of February, 2018 with the prevalence of congenial weather

conditions such as relatively high relative humidity of 52.8 per cent accompanied by slight rain (3.4 mm), maximum temperature of 22.12 °C and minimum temperature of 7.6°C where maximum incidence was seen during 2<sup>nd</sup> and 3<sup>rd</sup> week of April, 2018 due to the presence of suitable factors responsible to cause severe epiphytotic. Van Doorn (1959) has reported that 95 per cent or more relative humidity, at least for 11 h followed by RH level above 80%, were required for mildew outbreaks. Survivability of sporangia is dependent on temperature, RH and especially, the absence of strong radiation. The temperature optimal for epidemics is 10-12 °C but temperatures up to approx. 22 °C can also support rapid disease development under field (Palti, 1989).

#### **4.6 DISEASE MANAGEMENT**

##### **4.6.1 *In vitro* efficacy of fungicides on sporangial germination inhibition and germ tube length**

In order to study the effect of different fungicides on the sporangial germination inhibition and germ tube length, seven fungicides were tested against the *P. destructor* by spore germination method and the data pertaining to these parameters were recorded and presented in Table 4.10. Results in inhibition of sporangial germination by different concentration of the test fungicides (non-systemic and combi-products) at different time intervals is presented in Table 4.10. From the present investigation, it was observed that with a progressive increase in fungicide concentration there was a corresponding increase in inhibition of sporangial germination and the advancement of time period has caused a corresponding decrease in inhibition of sporangial germination. Among the non-systemic fungicides Dithane M-45 was the most effective showing almost complete inhibition in germination of sporangia at 750 and 1000 ppm from beginning 12 hours to 36 hours of observation. Similar trends were followed with respect to the combi-products, Ridomil Gold and Cabrio Top while Antracol, Kavach and Nativo resulted in cent per cent sporangial inhibition at highest tested concentration of 1000 ppm. Blitox 50 was least effective fungicide in controlling the complete inhibition of sporangial germination at all 500, 750 and 1000 ppm. Fungicides in particular were more efficacious and superior at higher concentrations. Cabrio Top, Nativo were next best fungicides followed by Antracol and Kavach at all time intervals in reducing the spore germination, though Nativo, Antracol and Kavach showed good effect only at higher concentrations i.e. 500 and 1000 ppm. At lower concentration of 200 ppm, Nativo appeared least effective compared to tested fungicides by giving 53.16 % sporangial germination inhibition after 12 hours while after 36 hours it was recorded to be

78.27 per cent but found statistically superior to the non-systemic fungicides at 500 ppm even after 36 hours of observation.

The results related to germ tube length of the pathogen, *Peronospora destructor* presented in Table 4.11 revealed that the Ridomil Gold (300 ppm) was statistically superior to all the fungicides tested and was effective in controlling germ tube length followed by Cabrio Top (300 ppm) and Dithane M-45 at 500 ppm, at all the three time intervals (12, 24, 36 hours). Blitox 50 was least effective in suppressing the germ tube even at 1000 ppm. All the fungicides tested did not yield any germ tube formation at higher concentration of 1000 ppm, followed by 750 ppm in case of non-systemic fungicides. Concentration of 300 ppm inhibited the sporangial germination with respect to combi-products, Ridomil Gold, Cabrio Top at all the specified time period. Maximum germ tube length (8.40  $\mu\text{m}$ ) reduction was obtained in Ridomil Gold followed by Cabrio Top (8.90  $\mu\text{m}$ ), Dithane M-45 among non-systemic fungicide (9.40  $\mu\text{m}$ ) after 12 hours thereafter a slight decrease in germ tube length was recorded as time interval advances and reached to 36 hours of incubation. Sporangia of *Peronospora destructor* has shown maximum sensitivity in response to Ridomil Gold, that is well supported by many workers where Blitox-50 and Chlorothalonil was found moderately effective in controlling sporangial germination (Wilson 1980; Boyadzhiev *et al.*, 1982; Jespersen and Sutton 1987; Sugha and Singh, 1991; Develash and Sugha, 1997 a)

#### **4.6.2 Efficacy of SAR chemicals and different fungicides under *in vivo* conditions**

Field trial data (Table 4.12) on the efficacy of five best fungicides obtained from *in vitro* evaluation and three SAR chemicals against downy mildew of onion showed that all the treatments were significantly superior in terms of effectiveness in reducing the disease compared with the untreated plots. During the crop season (2017-18), Chitosan, Cabrio Top, Potassium orthophosphate, Ridomil Gold and Dithane M-45 all differed significantly in their effect in checking the downy mildew infection. Maximum (81.93 %) disease control was achieved in Chitosan with minimum disease severity of 12.52 per cent while in other effective treatments, the per cent disease control ranged between 71.84 to 79.56. Effect of plant height was also taken into consideration in this research work, where it was noted that the chemicals that have supported good control over disease have also provided improvement in plant height, maximum (43.89 cm) plant height being recorded in case of Chitosan treatment. This was followed by Ridomil Gold (40.06 cm) in comparison to other tested SAR chemicals, fungicides and control (untreated plants) where only 27.72 cm average growth of the plants was obtained.

**Table 4.10 Effect of different fungicides on sporangial germination inhibition (%) of *Peronospora destructor***

Fungicides	After 12 hours			Mean	After 24 hour			Mean	After 36 hours			Mean
	Sporangial inhibition (%)				Sporangial inhibition (%)				Sporangial inhibition (%)			
	500	750	1000		500	750	1000		500	750	1000	
<b>Dithane M-45 (Mancozeb 75% WP)</b>	75.82 (50.97)	100 (71.54)	100 (71.54)	<b>91.94</b> <b>(64.68)</b>	70.98 (49.13)	100 (71.54)	100 (71.54)	<b>90.32</b> <b>(64.07)</b>	69.05 (49.01)	100 (71.54)	100 (71.54)	<b>89.68</b> <b>(64.03)</b>
<b>Blitox 50(Copper oxychloride 50% WP )</b>	65.62 (47.35)	82.35 (53.69)	96.3 (62.28)	<b>81.42</b> <b>(54.44)</b>	58.6 (44.88)	79.75 (52.12)	95.13 (61.27)	<b>77.83</b> <b>(52.76)</b>	57.87 (44.47)	78.67 (51.75)	94.93 (60.57)	<b>77.16</b> <b>(52.26)</b>
<b>Kavach (Chlorothalonil 75% WP)</b>	70.8 (49.04)	88.79 (57.27)	100 (71.54)	<b>87.03</b> <b>(59.28)</b>	68.8 (48.42)	86.92 (56.17)	100 (71.54)	<b>85.24</b> <b>(58.71)</b>	67.95 (47.85)	78.24 (52.63)	100 (71.54)	<b>82.06</b> <b>(57.34)</b>
<b>Antracol (Propineb 70% WP)</b>	70.82 (49.25)	90.3 (58.42)	100 (71.54)	<b>87.04</b> <b>(59.74)</b>	67.1 (48.07)	89.73 (57.25)	100 (71.54)	<b>85.61</b> <b>(58.95)</b>	66.97 (47.82)	88.34 (57.37)	100 (71.54)	<b>85.10</b> <b>(58.91)</b>
<b>Combi-Product</b>	<b>200</b>	<b>300</b>	<b>500</b>		<b>200</b>	<b>300</b>	<b>500</b>		<b>200</b>	<b>300</b>	<b>500</b>	
<b>Ridomil Gold 68 WG (Mancozeb 64% + Metalaxyl 4%)</b>	88.68 (57.44)	100 (71.54)	100 (71.54)	<b>96.23</b> <b>(77.25)</b>	86.82 (56.35)	100 (71.54)	100 (71.54)	<b>95.60</b> <b>(66.48)</b>	85.79 (55.78)	100 (71.54)	100 (71.54)	<b>95.26</b> <b>(66.29)</b>
<b>Nativo 75 WG (Tebuconazole 50%+ Trifloxystrobin 25%)</b>	73.16 (51.51)	82 (63.44)	100 (71.54)	<b>85.05</b> <b>(62.16)</b>	72.97 (48.64)	86.7 (56.24)	100 (71.54)	<b>86.56</b> <b>(58.8)</b>	72.45 (48.24)	96.33 (55.55)	100 (71.54)	<b>89.59</b> <b>(58.44)</b>
<b>Cabrio Top 60 WG (Pyraclostrobin 5% + Metiram 55%)</b>	85.7 (56.54)	100 (71.54)	100 (71.54)	<b>95.23</b> <b>(66.54)</b>	84.00 (55.46)	100 (71.54)	100 (71.54)	<b>94.67</b> <b>(66.18)</b>	83.32 (55.18)	100 (71.54)	100 (71.54)	<b>94.44</b> <b>(66.09)</b>
<b>Mean</b>	<b>75.8</b> <b>(51.73)</b>	<b>91.92</b> <b>(63.92)</b>	<b>99.47</b> <b>(70.22)</b>	<b>89.06</b> <b>(61.96)</b>	<b>72.75</b> <b>(50.14)</b>	<b>91.87</b> <b>(62.34)</b>	<b>99.30</b> <b>(70.07)</b>	<b>87.97</b> <b>(60.85)</b>	<b>71.91</b> <b>(49.76)</b>	<b>91.65</b> <b>(61.70)</b>	<b>99.27</b> <b>(69.97)</b>	<b>87.61</b> <b>(60.45)</b>
<b>CD<sub>0.05</sub></b>				<b>Fungicides(F)= 0.18</b>								
				<b>Concentration (C)= 0.12</b>								
				<b>Time (T)= 0.12</b> <b>F x C x T= 0.53</b>								

\* Figures in the parentheses are arc sine transformed values

**Table 4.11 Effect of different fungicides on germ tube length ( $\mu\text{m}$ ) production of *Peronospora destructor***

Fungicide Contact Conc.(ppm)	After 12 hours			Mean	After 24 hour			Mean	After 36 hours			Mean
	Germ tube length ( $\mu\text{m}$ )				Germ tube length( $\mu\text{m}$ )				Germ tube length( $\mu\text{m}$ )			
	500	750	1000		500	750	1000		500	750	1000	
<b>Dithane M-45 (Mancozeb 75% WP)</b>	28.21 (32.07)	00.00 (0.00)	00.00 (0.00)	<b>9.4</b> <b>(10.69)</b>	34.76 (36.71)	00.00 (0.00)	0.00 (0.00)	<b>11.5</b> <b>(12.23)</b>	35.19 (37.14)	00.00 (0.00)	00.00 (0.00)	<b>11.7</b> <b>(12.38)</b>
<b>Blitox 50(Copper oxychloride 50% WP )</b>	40.63 (39.59)	29.09 (31.35)	17.69 (24.86)	<b>28.47</b> <b>(19.85)</b>	49.25 (43.98)	37.49 (33.88)	18.21 (28.11)	<b>34.98</b> <b>(35.32)</b>	49.66 (44.23)	37.89 (34.27)	18.65 (28.51)	<b>35.39</b> <b>(35.67)</b>
<b>Kavach (Chlorothalonil 75% WP)</b>	34.66 (36.05)	26.93 (23.52)	0(0)	<b>16.87</b> <b>(21.28)</b>	40.06 (39.84)	27.53 (25.93)	00.00 (0.00)	<b>22.53</b> <b>(21.92)</b>	40.46 (40.2)	27.93 (26.25)	00.00 (0.00)	<b>22.73</b> <b>(22.15)</b>
<b>Antracol (Propineb 70% WP)</b>	38.20 (38.16)	28.79 (25.68)	00.00 (0.00)	<b>19.0</b> <b>(21.28)</b>	45.74 (42.54)	30.40 (29.59)	00.00 (0.00)	<b>25.38</b> <b>(24.04)</b>	46.18 (42.84)	30.83 (30.01)	00.00 (0.00)	<b>25.68</b> <b>(24.28)</b>
<b>Combi-product</b>	<b>200</b>	<b>300</b>	<b>500</b>		<b>200</b>	<b>300</b>	<b>500</b>		<b>200</b>	<b>300</b>	500	
<b>Ridomil Gold 68 WG (Mancozeb 64% + Metalaxyl 4%)</b>	25.22 (26.71)	0.00 (0.00)	0.00 (0.00)	<b>8.4</b> <b>(8.90)</b>	27.27 (31.47)	00.00 (0.00)	00.00 (0.00)	<b>9.09</b> <b>(10.49)</b>	27.66 (31.89)	00.00 (0.00)	00.00 (0.00)	<b>9.22</b> <b>(10.63)</b>
<b>Nativo 75 WG (Tebuconazole 50%+ Trifloxystrobin 25%)</b>	29.82 (28.52)	18.57 (27.09)	0.00 (0.00)	<b>13.8</b> <b>(18.53)</b>	30.2 (33.32)	24.763 (27.09)	00.00 (0.00)	<b>18.98</b> <b>(20.13)</b>	30.62 (33.72)	25.13 (27.51)	00.00 (0.00)	<b>18.58</b> <b>(20.41)</b>
<b>Cabrio Top 60 WG (Pyraclostrobin 5% + Metiram 55%)</b>	26.93 (29.28)	0.00 (0.00)	0.00 (0.00)	<b>8.90</b> <b>(9.76)</b>	28.193 (32.515)	00.00 (0.00)	00.00 (0.00)	<b>9.39</b> <b>(10.83)</b>	28.613 (32.95)	0 (0)	0 (0)	<b>9.53</b> <b>(10.98)</b>
<b>Mean</b>	<b>31.96</b> <b>(32.91)</b>	<b>14.77</b> <b>(15.37)</b>	<b>2.52</b> <b>(3.55)</b>		<b>36.50</b> <b>(37.20)</b>	<b>17.17</b> <b>(16.64)</b>	<b>3.60</b> <b>(4.01)</b>		36.91 (37.57)	17.39 (16.87)	2.66 (4.07)	

**CD<sub>0.05</sub> = Fungicides(F)=0.15, Concentration (C)= 0.09, Time (T)=0.09**

**F x C x T= 0.44**

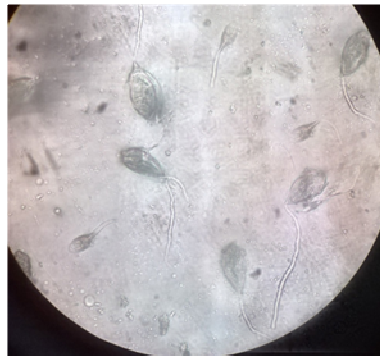
\* Figures in the parentheses are arc sine transformed values

Chitosan being maximum effective, possessed maximum growth, gave excellent control of disease and supported maximum bulb yield (26.7 t/ha) followed by Ridomil Gold (24.5 t/ha) and Cabrio Top (23.3 t/ha), the latter two were however statistically at par in controlling the disease as well as supporting good bulb yield. Whereas Antracol followed by Nativo were least effective in controlling the disease as well as increasing the yield factor.

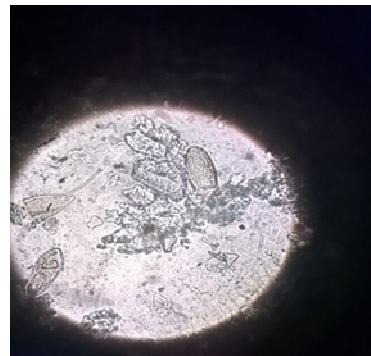
Hence, it is concluded from the results that the Chitosan not only checked the downy mildew to great extent but showed good ability in improving the plant height and bulb yield. Chitosan nanoparticles have got higher degree of acetylation that induces resistance against pearl millet downy mildew through nitric oxide generation (Siddaiah *et al.*, 2018).

Other fungicides next best in order were Ridomil Gold and Cabrio Top, both had equal ability in enhancing plant growth, managing the disease and have almost same yield in terms of the t/ha as the two were reported to be statistically at par with each other. Chitosan is an active inducer of defense responses against downy mildew disease and thus has the potential of becoming an alternative means of disease control (Sharathchandra *et al.*, 2004.) The efficiency of phosphite application in certain pathosystems is due to the fact that the plant has better assimilation in the presence of phosphorus and potassium, making it able to activate defence mechanisms and to produce phytoalexins, natural self-defence substances that confer resistance against pathogens especially in grapevine downy mildew system. Phytogard ( $K_2HPO_3$ ) may constitute an alternative method to counter downy mildew of Brassicas (Bécot *et al.*, 2000; Jackson *et al.*, 2000; Nojosa *et al.*, 2005). Phosphonate or potassium derivatives such as  $K_2HPO_4$  or  $KH_2PO_4$  induce SAR in different pathosystems. These compounds have similarities with  $K_2HPO_3$  (Phytogard) but they seem to have different modes of action (Reuveni and Reuveni, 1998). Post-inoculation application of BABA was reported to induce resistance against downy mildew of grape and *Phytophthora infestans*, tobacco from *Peronospora tabacina* (Cohen, 2002). Ridomil was effective when used @ 0.1% and applied 3 to 4 times fortnightly as compared to the untreated control treatment (Nasir *et al.*, 2015; Iqbal *et al.*, 2009; Raziq *et al.*, 2008). In another study conducted by Jones (1978) and Timchenko (1979), downy mildew of cucurbits and onion was effectively controlled by the use of Chlorothalonil which is contrary in the present study where as Blitox 50 did not give good result and hence should not be recommended for its control. Satou (2003) suggested various fungicides for the control of downy mildew in cucumber and other vegetables. Eleven fungicides were tested for the control of muskmelon downy mildew (*Pseudoperonospora cubensis*) and reported that Mancozeb (72% WP) stood first

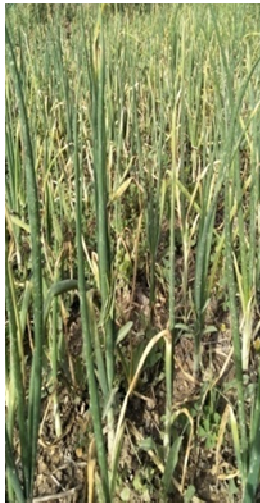
**Plate 5 Effect of fungicide on sporangial germination**



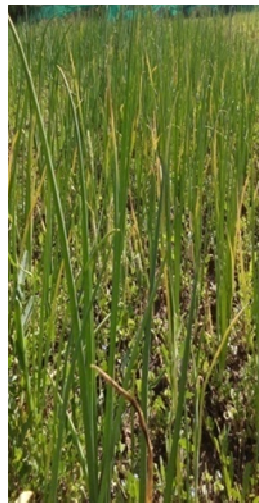
**Plate 5a Blitox at 500 ppm**



**Pate 5b Ridomil Gold at 500 ppm**



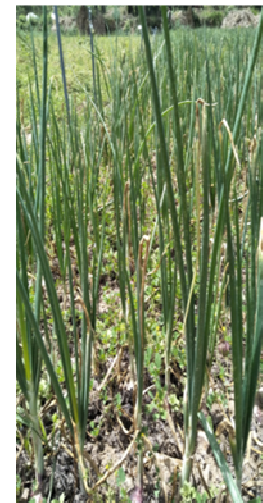
**Plate 6 a  
Control**



**Plate 6 b  
Blitox 50**



**Plate 6 c  
Chitosan**



**Plate 6 d  
Ridomil gold**

**Plate 6 In vivo evaluation of SAR and fungicides**

**Table 4.12 Effect of SAR and fungicides on onion downy mildew severity, plant height and bulb yield**

<b>Treatment</b>	<b>Conc. (%)</b>	<b>Disease severity (%)</b>	<b>Per cent disease control (%)</b>	<b>Height (cm)</b>	<b>Yield (kg/plot)</b>	<b>Yield (t/ha)</b>
<b>Chitosan</b>	0.05	12.5 (20.70)	81.9 (64.84)	43.9	4	26.7
<b>Potassium Orthophosphate</b>	0.20	17.9 (25.01)	74.2 (59.47)	36.1	3.33	22.2
<b>BABA (<math>\beta</math> amino butyric acid)</b>	0.10	22.4 (28.27)	67.7 (55.33)	31.5	2.67	17.8
<b>Dithane M-45 (Mancozeb 75% WP)</b>	0.25	19.6 (26.24)	71.8 (57.93)	33.1	3.17	21.1
<b>Antracol (Propineb 70% WP)</b>	0.3	32.9 (34.98)	52.7 (46.55)	32.4	3	20
<b>Ridomil Gold 68 WG (Mancozeb 64% + Metalaxyl 4%)</b>	0.25	14.5 (22.34)	79.2 (62.83)	40.1	3.67	24.5
<b>Cabrio Top 60 WG (Pyraclostrobin 5% + Metiram 55%)</b>	0.25	14.2 (22.13)	79.6 (63.09)	37.4	3.5	23.3
<b>Nativo 75 WG (Tebuconazole 50%+ Trifloxystrobin 25%)</b>	0.5	25.4 (30.28)	63.4 (52.74)	29.8	2.33	15.5
<b>Control</b>		69.5 (56.45)	0.00 (0.00)	27.7	2	13.3
<b>C.D<sub>0.05</sub></b>		1.25	1.55	1.58	0.94	

\* Figures in the parentheses are arc sine transformed values

showing minimum disease incidence of 3.16 per cent (Anonymous, 2004). Other workers have also reported the efficacy of Ridomil and Dithane M-45 in controlling the downy mildews (Sharma *et al.*, 2003; Chaudhary *et al.*, 2009; Bhat *et al.*, 2018). The studies on the management of downy mildew disease of bittergourd has shown that the Pyraclostrobin was found best in controlling the downy mildew where Propineb was the least effective (Vijayaraghavan *et al.*, 2017). Survielliené *et al.* (2008) and González-Rodríguez *et al.* (2009) has reported the efficacy of strobilurins in controlling the downy mildew disease of onion. Mo *et al.* (2016) reported Mancozeb as the best fungicide in order to control downy mildew. Fungicides containing Mancozeb and other active ingredients such as Benalaxyl, Dimetomorf, Metalaxyl are the most effective and result in more than 85% control of onion downy mildew (Hoppe BioEco, 2004). The highest efficacy against the downy mildew disease of onion was exhibited by Azoxystrobin + Chlorothalonil and Pyraclostrobin + Boscalid, which has performed efficiently in preventing infection with storage diseases (over 90%), and had a beneficial effect on the storage life of these vegetable roots (Robak and Adamicki, 2007).

Plots treated with fungicides has produced marketable onion bulb yield and it confirms that early defoliation of onion due to downy mildew has reduced bulb sizes and caused yield losses.( Lorbeer and Andaloro, 1984; Develash and Sugha, 1997 b; Gilles *et al.*, 2004; Gianessi and Reigner, 2005; Surviliené *et al.*, 2008). For getting a better control of downy mildew in onion it is important to make prophylactic sprays as soon as the first disease symptoms appear in the field.

The maximum yield in the fungicide treated plots may be due to the more number of leaves, maximum plant height and less disease severity that indirectly contributes to the higher yields (Raziq *et al.*, 2008; Iqbal *et al.*, 2009).

## Chapter-5

# SUMMARY AND CONCLUSION

---

The present investigation on onion downy mildew (*Peronospora destructor* (Berk.) Casp. ex Berk) as undertaken with respect to occurrence, identification, pathogenicity, epidemiological studies and management of disease through chemicals and SAR inducers. The results obtained are summarized below:

Onion downy mildew was low, moderately and severely prevalent in all onion growing areas of Solan district. Maximum disease incidence (39.24 %) and severity (58.65%) of downy mildew of onion was observed at Pandah and followed by Khaltoo during the cropping season of 2017-2018 and mean per cent incidence and disease severity of downy mildew were recorded to be 23.71 and 25.34, respectively at all other locations surveyed of Solan district.

Characteristic symptoms seen on leaves were initially circular or elliptical lesions that become white, slightly sunken and roughened and with the passage of time these lesions were covered with the grey downy masses that turned purplish after heavy rain. In case of severe downy mildew infestation, necrosis followed by girdling was also seen. Few bottleneck bulbs were seen due to severe infection of aerial portions of the plant. Downy mildew pathogen has also affected the bulb development and reduced the yield, as infected bulbs were smaller than healthy bulbs.

On the basis of appearance and morphological characteristics of the pathogen like nature of mycelium which observed as hyaline, aseptate with a width of 3.6-6.9  $\mu\text{m}$  and characteristic dichotomously branched sporangiophores bearing pyriform sporangia measuring 50.4-65.8 X 24.6-25.2  $\mu\text{m}$  in size. Based on the morphological characters, the pathogen was identified as *Peronospora destructor* (Berk.) Casp. ex Berk.

In order to prove the pathogenicity, the pathogen was inoculated by following two methods detached leaf and spray and drop inoculation with sporangial suspension and typical symptoms similar to the original symptoms developed after 8 days and 12-14 days of inoculation of the pathogen, respectively.

Epidemiological studies of pathogen revealed that pathogen preferred temperature of 15°C as optimum for sporangial germination and the favourable relative humidity was above 95.1 per cent with maximum sporangial germination at 100 per cent. Disease initiated when favourable conditions like temperature and relative humidity prevailed for at least two days.

Under field conditions disease appeared at the end of February 2018, due to the presence of favourable mean maximum temperature (22.12 °C), mean minimum temperature (7.6 °C) and average relative humidity (52.8 %) and attained maximum severity (72.76 %) during second week of April, 2018. The simple and partial correlation were highly significant and positively correlated with mean maximum temperature, mean minimum temperature, average relative humidity and negative with rainfall. Multiple correlation analysis has shown that 87.4 per cent disease severity was due to the cumulative effect of all the factors considered under the study.

Among different fungicides tested under *in vitro* conditions, four non-systemic fungicides evaluated against the *Peronospora destructor* revealed that Dithane M-45 resulted in effective sporangial germination inhibition at all concentrations up to 91.94 per cent whereas 75.82 at lowest concentration of 500 ppm followed by Kavach and Antracol giving 70.00 per cent sporangial germination inhibition at 500 ppm concentration. Blitox was found least effective with 65.62 per cent sporangial germination inhibition at 500 ppm concentration. Out of three Combi-products evaluated against the pathogen under *in vitro* conditions, Ridomil Gold at 200ppm and Cabrio Top at 200 ppm were found most effective and significantly superior over other treatments at 300 and 500 ppm, respectively. However, Nativo was least effective at all the concentrations. In general higher concentrations of chemicals has shown maximum inhibition of sporangial germination and germ tube length. Germ tube length was also reduced to great extent in case of Ridomil Gold (8.40 µm) after 12 hours of incubation followed by Cabrio Top and Dithane M-45 at all the time intervals.

Similarly, field evaluation of fungicides and SAR chemicals as foliar sprays against downy mildew of onion showed that Chitosan was found superior over other treatments followed by Ridomil Gold and Cabrio Top by giving disease reduction in the range of 79.56 to 81.93 per cent. Minimum disease severity however was recorded with foliar sprays of Antracol followed by Nativo. Foliar sprays of Chitosan was

supported by maximum height (43.89 cm) and highest yield (26.7 t/ha) followed by Ridomil Gold (40.06 cm, 24.5 t/ha) and Cabrio Top (37.36 cm, 23.3 t/ha).

From the above discussion it is concluded that downy mildew of onion is a serious disease in low lying areas of Solan district of Himachal Pradesh and was caused by an oomycete *Peronospora destructor* (Berk.) Casp. ex Berk. The congenial temperature was found to be 15°C and relative humidity more than 95 per cent for its development. Three protective sprays of Chitosan, a SAR chemical (at 3-4 leaf stage followed by next two sprays after the appearance of disease), Ridomil Gold and Cabrio Top at 15 days interval on the onset of disease was observed to manage the downy mildew disease effectively and could be recommended for its management under farmers' field.

# LITERATURE CITED

---

- Abd-Elrazik AA and Lorbeer JW. 1980. A procedure for isolation and maintenance of *Peronospora destructor* on onion. *Phytopathology* 70:780-82.
- Agostini JP, Bushong PM and Timmer LW. 2003. Green house evaluation of products that induce host resistance for control of scab, melanose and Alternaria brown spot of citrus. *Plant Disease* 87:69-74.
- Agrios GN. 1995. *Plant Pathology*. 2<sup>nd</sup> edition. Academic Press, New York. 838 p.
- Ahmad S and Hakim K. 2000. Effects of fungicides synergy on downy mildew control in onion. *Pakistan Journal of Biological Sciences* 3:1042-43.
- Akram W and Anjum T. 2011. Use of bioagents and synthetic chemicals for induction of systemic resistance in tomato against diseases. *International Journal of Agricultural Science and Soil Science* 1:286-92.
- Alexopoulos CJ, Mims CW and Blackwell M. 1962. *Introductory Mycology*. 2<sup>nd</sup> edition. John Wiley & Sons, New York. 869p.
- Anonymous. 1982. *Annual Report*. Horticultural Crops Development Authority (H.C.D.A) Kenya. 35p.
- Anonymous. 1999. *WHO Monographs on Selected Medicinal Plants*. World Health Organization, Geneva. 12p.
- Anonymous. 2004. *Annual Report (2003-2004)*. Ayub Agriculture Research Institute Faisalabad. 178p.
- Anonymous. 2005. *Cultivation of cauliflower*. <http://www.growingformarket.com>. [11.30 PM, 10th February, 2018]
- Anonymous. 2015. *History of onion*. <http://herbalextractsplus.com/onion.cfm>) [11:00 AM, 12th January, 2018]
- APEDA. 2017. <http://apeda.gov.in/apedawebsite/> [11.30 PM, 10<sup>th</sup> September, 2017]
- Arita E and Santos J. 2012. "Burned tip" or downy mildew in onions. Plantwise factsheet for farmers, CABI. <http://www.plantwise.org>. [12.30 PM, 26th March, 2017]
- Armstrong GM and Sumner CB. 1935. *Investigations of downy mildew of tobacco*. Bull. S.C. Agriculture Experiment Station. 303p.
- Bashi E and Aylor DE. 1983. Survival of detached sporangia of *Peronospora destructor* and *Peronospora tabacina*. *Phytopathology* 73:1135-39.
- Baswana KS. 1984. Role of insect pollination on seed production in coriander and fennel. *South Indian Horticulture* 32:117-18.
- Bécot S, Pajot E, Le Corre D, Monot C and Silué D. 2000. Phytogard (K<sub>2</sub>HPO<sub>3</sub>) induces localized resistance in cauliflower to downy mildew of crucifers. *Crop Protection* 19:417-25.

- Ben-shalom N, Ardi R, Pinto R, Aki C and Fallik E. 2003. Controlling gray mold caused by *Botrytis cinerea* in cucumber plants by means of chitosan. *Crop Protection* 22:285–90.
- Bhaskarareddy MV, Arul J, Angers P and Couture L. 1999. Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality. *Journal of Agricultural and Food Chemistry* 47:1208–16.
- Bhat JA, Rashid R, Dar WA and Bhat RA. 2018. Efficacy of different fungicides for the management of downy mildew of cucumber grown under low plastic tunnel. *International Journal of Pure and Applied Biosciences* 6:884-90.
- Bounaurio R, Scarponi L, Ferrera M, Sidott P and Bertona A. 2002. Induction of systemic acquired resistance in Acibenzolar-S-methyl against bacterial spot disease. *European Journal of Plant Pathology* 108:41–49.
- Boyadzhiev K, Angelov D and Vitanov M. 1982. Chemical control trials against downy mildew (*Peronospora destructor* [Berk.] Casp.) on onion bulbs. *Gradin. Loz. Nauka* 19: 75-80.
- Brewster JL. 2001. The structure of edible alliums. In: *Onions and other allium* (López M and Brewster JL eds). Editorial Acribia, Zaragoza. pp.230-266.
- Butler JE and Jones SD. 1955. *Plant Pathology*. Macmillan and Co., New York. 693 p.
- Chaudhry SU, Iqbal J and Mustafa A. 2009. Efficacy of different fungicides for the control of downy mildew of cucumber. *Journal of Animal and Plant Sciences* 19:202-04.
- Chen Y and Wang D. 2005. Two convenient methods to evaluate soybean for resistance to *Sclerotinia sclerotiorum*. *Plant Disease* 89:1268–72.
- Chupp C and Sherf AF. 1960. *Vegetable Diseases*. The Ronald Press Company, New York. 553p.
- Cohen Y and Eyal H. 1977. Growth and differentiation of sporangia and sporangiophores of *Pseudoperonospora cubensis* on cucumber cotyledons under various combinations of light and temperature. *Physiological Plant Pathology* 10: 93-95.
- Cohen Y and Sherman Y. 1977. The role of air-borne conidia on the epiphytotics of *Sclerospora sorghi* on sweet corn. *Phytopathology* 66: 515-21.
- Cohen Y, Eyal H and Sadon T. 1975. Light-induced inhibition of sporangial formation of *Phytophthora infestans* on potato leaves. *Canadian Journal of Botany* 53: 2680-86.
- Cohen Y. 1977. The interacting effects of light and temperature on sporulation of *Peronospora tabacina* on tobacco leaves. *Australian Journal of Biological Science* 29:281-89.
- Cohen Y. 2002.  $\beta$ -aminobutyric acid induced resistance against plant pathogens. *Plant Disease* 86: 448-57.
- Cohen Y, Vaknin M, Ben-Naim Y and Rubin AE. 2013. Light suppresses sporulation and epidemics of *Peronospora belbahrii*. *PLoS one* 8: e81282.

- Conrath U, Beckers GJ, Flors V, García-Agustín P, Jakab G and Mauch F. 2006. Priming: getting ready for battle. *Molecular Plant-Microbe Interaction* 19: 1062-71
- Cook HT. 1932. *Downy mildew disease of onion*. Mem. Agric. Exp. Station, Cornell, New York. 40p.
- Cynthia M and Ocamb M. 2008. *Onion (Allium cepa) downy mildew disease*. An online guide to plant diseases. Oregon State University Extension service, USA.
- Dercks W and Creasy LL. 1989. Influence of fosetyl-Al on phytoalexin accumulation in the *Plasmopara viticola*-grapevine interaction. *Physiological and Molecular Plant Pathology* 34:203–13.
- De Visser CLM. 1998. Development of a downy mildew advisory model based on downcast. *European Journal of Plant Pathology* 104:933-43.
- Decapdiville G, Beer SV, Watkins CB, Charles L, Wilson CL, Luis O and Tedeshi LO. 2003. Pre and post-harvest harpin treatment of Apples induce resistance to blue mold. *Plant Disease* 87:39–44.
- Develash RK and Sugha SK. 1997 a. Management of downy mildew (*Peronospora destructor*) of onion (*Allium cepa*). *Crop Protection* 16:63–67.
- Develash RK and Sugha SK. 1997 b. Incidence of downy mildew and its impact on yield. *Indian Phytopathology* 50:127–29.
- Domingues RJ and Töfoli JG. 2009. *Onion mildew: Importance, identification and control methods*. Technical Disclosure 71. Biological Institute, São Paulo. 31p.
- Dubreuil-Maurizi C, Trouvelot S, Frettinger P, Pugin A, Wendehenne A and Poinssot B. 2010.  $\beta$ -aminobutyric acid primes an NADPH oxidase-dependent reactive oxygen species production during grapevine-triggered immunity. *Molecular Plant-Microbe Interactions* 23:1012–21.
- El Hadrami A, Adam LR, El Hadrami I and Daayf F. 2010. Chitosan in plant protection. *Marine Drugs* 8:968–87.
- FAO. 2016. Food and agriculture data. <http://www.fao.org/faostat/en/#data> [2.00 PM, 15th November, 2017]
- Farr DF and Rossman AY. 2015. *Fungal databases, systematic mycology and microbiology laboratory*. ARS, USDA. [http://nt.ars-grin.gov/fungal\\_databases/](http://nt.ars-grin.gov/fungal_databases/) [10.30 AM, 18th March, 2018]
- Georgy NI, Radwan JA, Mohamed HA and Shabi AE. 1986. Chemical control of downy mildew and purple blotch of onion in Egypt. *Agric. Res. Rev.* 61:25-41.
- Gianessi LP and Reigner N. 2005. *The value of fungicides in US crop production*. Crop Life Foundation, Crop Protection Research Institute, Washington, DC. 243p.
- Gilles T, Phelps K, Clarkson JP and Kennedy R. 2004. Development of MILIONCAST, an improved model for predicting downy mildew sporulation on onions. *Plant Disease* 88: 695–702.

- Godard S, Slacanin I, Viret O and Gindro K. 2009. Induction of defence mechanisms in grapevine leaves by emodin- and anthraquinone- rich extracts and their conferred resistance to downy mildew. *Plant Physiology and Biochemistry* 47:827-37.
- Göker M, Voglmayr H, Riethmüller A and Oberwinkler F. 2007. How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews. *Fungal Genetics and Biology* 44:105-22.
- Golyshin NM, Maslova AA and Goncharova TF. 1991. Systemic fungicides as protection of onion seed plants against downy mildew. *Agrokimiya* 9:115-17.
- Gomez KA and Gomez AA. 1984. *Statistical Procedures for Agricultural Research*. 2<sup>nd</sup> ed. John Wiley and Sons Inc, New York. 680p.
- Goncalves PA, deSousa E, Silva CR and Boff P. 2004. Incidence of downy mildew in onion growing under mineral and organic fertilization. *Horticulture Brasileira* 22: 538–42.
- González PH, Colnago P, Peluffo S, Idiarte HG, Zipitría J and Galván GA. 2010. Quantitative studies on downy mildew (*Peronospora destructor* Berk. Casp.) affecting onion seed production in southern Uruguay. *European Journal of Plant Pathology* 129:303-14.
- González-Rodríguez RM, Cancho-Grande B and Simal-Gándara J. 2009. Efficacy of new commercial formulations to control downy mildew and dissipation of their active fungicides in wine after good agricultural practices. *Journal of the Science of Food and Agriculture* 89: 2625-35.
- Gupta RP, Srivastava PK and Pandey UB. 1984. Downy mildew of onion (*Allium cepa* L.) - A new disease from plains of India. *Indian Journal of Plant Pathology* 2:77-82.
- Gupta VK and Paul YS. 2001. *Diseases of Vegetable Crops*. Kalyani Publisher, New Delhi. 223p.
- Hadwiger LA. 1999. Host-parasite interactions: elicitation of defense responses in plants with chitosan. In: *Chitin and Chitinases* (Jolles P and Muzzarelli RAA eds). Birkhauser Verlag, Switzerland. pp.185–200.
- Hadwiger LA. 2013. Multiple effects of chitosan on plant systems: solid science or hype. *Plant Science* 208:42–49.
- Hakim K, Gill MA and Ihsan J. 2002. Chemical control of downy mildew of onion. *Pakistan Journal of Phytopathology* 14:91-94.
- Heil M and Bostock RM. 2002. Induced systemic resistance against pathogens in the context of induced plant defense responses. *Annals of Botany* 89:503–12.
- Hilderband PD and Sutton JC. 1982. Weather variables in relation to an epidemic of onion downy mildew. *Phytopathology* 72:219-24.
- Hildebrand PD and Sutton JC. 1984. Interactive effects of the dark period, humid period, temperature, and light on sporulation of *Peronospora destructor*. *Phytopathology* 74:444–49.
- Hiura M.1930. Studies on some downy mildews of agricultural plants. IV. On the downy mildew of the Welsh onion. (Report II.) *Agr. and Hort. [Tokyo]* 5:1008-14

- Hoffmann MP, Petzoldt CH and Frodsham AC. 1996. *Integrated Pest Management for Onions*. New York State IPM Program Publication, New York. 119p.
- Hoppe BioEco. 2004. [http://www.aametica.com/Kem-Kol\\_results\\_Fruit\\_Veges.pdf](http://www.aametica.com/Kem-Kol_results_Fruit_Veges.pdf)
- Ikata S and Yamauti K. 1941. Notes on the haustoria of some species of *Peronospora*. *Annual Phytopathological Society of Japan* 10:326-28.
- Iqbal M , Sahi GM , Tahir HAS, Sahi ST and Atif M. 2009. Evaluation of different fungicides against downy mildew of onion. *Pakistan Journal of Phytopathology* 21:104-07.
- Jackson TJ, Jones GM and Steven FGL. 2000. Action of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*. *Plant Pathology* 49: 147–54.
- James WC. 1974. Assessment of plant disease and losses. *Annual Review of Phytopathology* 12:27-48.
- Jayakumar M, Ponnuswamy K and Amanullah MM. 2008. Influence of nitrogen and intercropping on pest incidence, yield attributes and yield of cotton. *Journal of Applied Scientific Research* 4: 224-28.
- Jespersion G and Sutton JC. 1987. Evaluation of a forecaster for downy mildew of onion (*Allium cepa* L.). *Crop Protection* 6: 95-103.
- Jones HA. 1932. Breeding for resistance to onion downy mildew caused by *Peronospora destructor*. *Hilgardia* 12:531-50.
- Jones JP. 1978. Disease threshold for many downy mildew and target leaf spot of cucurbits and late blight of tomato. *Plant Disease Reporter* 62: 689-702.
- Jones R. 2000. *Formulating New Options*. Fruit Grower, Meister Publications, Boston, USA.
- Jordan RW. 1915. *Onions*. Webb Publishing Co., St. Paul, Minnesota. 95p.
- Jovicevic BM. 1964. Researches on biology, ecology and control of the onion parasite *Peronospora schleideni* Ung. in Siberia. *Zaštita Bilja* 77:167–72.
- Katerfeld NO. 1926. Contribution to the biology of *P. destructor*. *Morbi Plantarum* 15:71-77.
- Katiyar D, Hemantaranjan A, Bharti S and Nishant BA. 2014. A future perspective in crop protection: chitosan and its oligosaccharides. *Advances in Plants & Agriculture Research* 1:00006.
- Kennedy R. 1998. *Bulb onions: Evaluation of fungicides for control of downy mildew (Peronospora destructor)*. Horticultural Development Council, Annual Report (Year 1) for project, FV.10p.
- Kessmann H, Staub T, Hoffmann C, Maetzke T, Herzog J and Ward E.1994. Induction of systemic acquired disease resistance in plants by chemicals. *Annual Review of Phytopathology* 32:439–59.
- Khan H and Ahmad S. 2001. Influence of host management on downy mildew control in onion. *Pakistan Journal of Biological Sciences* 4:1126–28.

- Kofoet A. 1986. Lichtmikroskopische und elektronenmikroskopische Untersuchungen zum Infektionsverlauf von *Peronospora destructor* an Zwiebeln. *Mitt. Biol. Bundesanst* 232:221-22.
- Kothari IL and Patel M. 2004. Plant immunization. *Indian Journal of Experimental Biology* 42: 244-52.
- Krauthausen HJ, Richter E, Hagner S and Hommes M. 2001. Epidemiology and control (based on thresholds) of leaf diseases (*Peronospora destructor*, *Botrytis* spp.) and thrips (*Thrips tabaci*) in onion. *Acta Horticulturae* 25: 137–40.
- Kuc J. 2001. Concepts and direction of induced systemic resistance in plants and its applications. *European Journal of Plant Pathology* 107: 7–12.
- Kumar PT. 2007. *Biological management of Alternaria blight of onion*. M.Sc Thesis. Department of Plant Pathology. University of Agricultural Sciences , Dharwad. 112p.
- LaMondia JA. 2009. Efficacy of fungicides and systemic acquired resistance activator (Acibenzolar-S-methyl) against tobacco blue mold. *Crop Protection*. 28:72e76.
- Langston DB, and Sumner DR. 2000. First report of downy mildew (caused by *Peronospora destructor*) of onion in Georgia. *Plant Disease* 84:489-91.
- Leach CM. 1985. Effect of still and moving moisture-saturated air on sporulation of *Drechslera* and *Peronospora*. *Transactions of British Mycological Society* 84:179-83.
- Lorbeer J and Andaloro J. 1984. *Diseases of Onions: Downy Mildew*. NYSAES, Geneva, New York.737p.
- Loss GD and Stenina NV. 1975. New toxic chemicals in control of downy mildew of onion. *Review of Plant Pathology* 54:478-82.
- Mandahar HK, Lyngs JHJ, Mathur SB and Smedegaard PV. 1998. Resistance to rice blast induced by ferric chloride, dipotassium hydrogen phosphate and salicylic acid. *Crop Protection* 17:323-29.
- Matari NH and Blair JE. 2014. A multilocus time scale for oomycete evolution estimated under three distinct molecular clock models. *BMC Evolutionary Biology* 14:101-09.
- Maude RB. 1990. Leaf diseases of onions. In: *Onion and allied crops* (Rabinowitch HD and Brewster JL eds). Boca Raton, CRC.pp.173–212.
- McKay R. 1939. Observations on onion mildew caused by the fungus *Peronospora schleideniana*. W. G. Sm. *Royal Horticultural Society Journal* 64:272-85
- McKinney HH. 1923. Influence of soil temperature and moisture on infection of wheat seedling by *Helminthosporium sativum*. *Journal of Agricultural Research* 26:195-217.
- Mersha Z. 2012. Evaluation of systemic acquired resistance inducers for control of downy mildew on basil. *Crop Protection* 40:83-90.

- Mir NM, Dhar AK, Khan MA, Dar GH and Zargar MY. 1987. Screening of fungicides for field control of downy mildew (*Peronospora destructor*) on onion. *Indian Journal of Mycology and Plant Pathology* 17: 321-22.
- Mirakhur RK, Dhar AK and Kaw MR. 1977. Downy mildew of *Allium cepa* and its control with fungicides in Kashmir valley. *Indian Phytopathology* 30:576-77.
- Mo CY, Lee JH , Ko SJ and Yang KY. 2016. Control efficacy of several fungicides against downy mildew of onion at nursery seedling stage. *Research in Plant Disease* 22: 208-12.
- Mohibullah.1991. *Studies on Major Diseases of Bulb Vegetables (onion and garlic) in N.F.W.P. Province, Pakistan: Final Technical Report (October 1986 to September 1991)*. Agricultural Research Institute, Tarnab (Peshawar), N.W.P. Pakistan.55p.
- Murphy P and Mckay R. 1926. The downy mildew of onions (*Peronospora schleideni*) with particular reference to the hibernation of the parasite. *The Scientific Proceedings of the Royal Dublin Society* 18:237-61.
- Nakov B. 1972. The effect of ecological conditions on the spread of downy mildew (*Peronospora parasitica*) in cabbage. *Nauchni Trudove, Vissh Selskostopanski Institut "Vasil Kolarov"* 21: 109-16.
- Nasir M, Iqbal B, Sajjad M, Idrees M, Mohy-Ud-Dina G, Iqra, Khan WA and Hannan A. 2015. Effectiveness of new fungicides against cucumber downy mildew under tunnel conditions. *Pakistan Journal of Phytopathology* 27:175-79.
- NHB. 2017. <http://nhb.gov.in/default.aspx> [2.10 PM, 15th November, 2017]
- Nojosa, GBA, Resende MLV and Resende AV. 2005. Uso de fosfitos e silicatos na indução de resistência. In: *Indução de resistência em plantas a patógenos e insetos* (Cavalcanti L and Resende AV eds). Piracicaba. pp.236-263.
- O'Herlihy EA, Duffy EM and Cassells AC. 2003. The effects of arbuscular mycorrhizal fungi and chitosan sprays on yield and late blight resistance in potato crops from plantlets. *Folia Geobotanica* 38:201-07.
- Oostendorp M, Kunz W, Dietrich B and Staub T. 2001. Induced disease resistance in plants by chemicals. *European Journal of Plant Pathology* 107:19-28.
- Palti J.1989. Epidemiology, prediction and control of onion downy mildew caused by *Peronospora destructor*. *Phytoparasitica* 17:31-48.
- Panse VG and Sukhatme PV. 2000. *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research, New Delhi, India. 378p.
- PDOGR. 2016. <http://www.dogr.res.in> [12.30 PM, 12<sup>th</sup> March,2018]
- Pieterse CM, Leon-Reyes A, Van der S and Van Wees SC. 2009. Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology* 5:308-16.
- Premila A and Sophiarani Y. 2015. Downy mildew of onion: epidemiological aspects. *International Journal of Innovative Research and Development* 4:13-15.
- Premila A. 2013. Fungal airspora over onion field in Manipur valley. *International Journal of Current Science & Technology* 2:177 – 79.

- Rakhimov UA and Sadygov AKH. 1975. Trial of chemical preparation against downy mildew on the Maselly varieties of onion in Lenkoran Zone. *Review of Plant Pathology* 54:378-80.
- Raziq F, Alam I, Naz I and Khan H. 2008. Evaluation of fungicides for controlling downy mildew of onion under field conditions. *Sarhad Journal of Agriculture* 24:85-92.
- Reddy MV, Asselin A and Arul J. 1997. Effect of chitosan on tissue maceration and enzyme production by *Erwinia carotovora* in Potato. *Horticultural Sciences* 32:511-13.
- Reuveni R and Reuveni M. 1998. Foliar fertilizer therapy – A concept in integrated pest management. *Crop Protection* 17:111-18.
- Reuveni R, Dor G, Rauveni M and Tuzum S. 2000. Systemic resistance against *S. fuliginea* in cucumber plants exposed to phosphate in hydroponics system and its control by foliar spray of mono-potassium phosphate. *Crop Protection* 19:355-61.
- Robak J and Adamicki F. 2007. *The effect of pre-harvest treatment with fungicide on the storage potential of root vegetables*. Vegetable Crops Research Bulletin. 67p.
- Randomanski, W.1967. *Studies on the epidemiology of onion downy mildew, Peronospora destructor* (Berk.) Fries. Technical Report for 1962–67. Research Institute for Vegetable Crops, Skierniewice, Poland. 59p.
- Ross IA. 2001. *Medicinal Plants of the world: Chemical Constituents, Traditional and Modern Medicinal Uses*. Vol 2. Humana Press, Totowa. 487p.
- Rumbolz J, Wirtz S, Kassemeyer HH, Guggenheim R and Schafer E. 2002. Sporulation of *Plasmopara viticola*: Differentiation and light regulation. *Plant Biology* 4: 413-22.
- Runge F, Choi YJ and Thines M. 2011. Phylogenetic investigations in the genus *Pseudoperonospora* reveal overlooked species and cryptic diversity in the *P. cubensis* species cluster. *European Journal of Plant Pathology* 129:135–46.
- Sangeetha CG and Siddaramaiah AL. 2007. Epidemiological studies of white rust, downy mildew and Alternaria blight of Indian mustard (*Brassica juncea* (Linn.) Czern. and Coss.) *African Journal of Agricultural Research* 2:305-08.
- Satou M. 2003. Recent topics on downy mildew of vegetables in Japan. *Agriculture Journal* 8:1-8.
- Schneider-Mueller S, Kurosaki F and Nishi A.1994 Role of salicylic acid and intracellular Ca<sup>2+</sup> in the induction of chitinase activity in carrot suspension culture. *Physiological and Molecular Plant Pathology* 45:101-09.
- Scholten OE, van Heusden AW, Khrustaleva LI, Burger-Meijer K, Mank RA, Antonise RG C, Harrewijn JL, van Haecke W, Oost EH, Peters RJ and Kik C. 2007. The long and winding road leading to the successful introgression of downy mildew resistance into onion. *Euphytica* 156:345-53.
- Schultz D and French RD. 2009. Texas AgriLife Extension Service (The Texas A&M System).<https://agriflifercdn.tamu.edu/amarillo/files/2010/11/DownyMildewOnion.pdf> [3.00 PM, 9th September, 2017]

- Schwartz HF and Mohan SK. 1995. *Compendium of Onion and Garlic Diseases*. American Phytopathological Society, St. Paul, MN.70p.
- Seidl MF, Van den Ackerveken G, Govers F and Snel B. 2011. A domain-centric analysis of oomycete plant pathogen genome reveals unique protein organization. *Plant Physiology* 155:628-44.
- Sharathchandra RG, Niranjana Raj S, Shetty NP, Amruthesh KN and Shekar Shetty H. 2004. A chitosan formulation Elexa induces downy mildew disease resistance and growth promotion in pearl millet. *Crop Protection* 23: 881–88.
- Sharma DR, Gupta SK and Shyam KR. 2003. Studies on downy mildew of cucumber caused by *Pseudoperonospora cubensis* and its management. *Indian Journal of Mycology and Plant Pathology* 33: 246-51.
- Sharma HC, Crouch JH, Sharma KK, Seetharama N and Hash CT. 2002. Applications of biotechnology for crop improvement: prospects and constraints. *Plant Sciences* 163:381-95.
- Sharma RC, Gill SS and Kohli N. 2002. Pathological problems in production and storage of onion seed in Punjab and their remedial measures. *Seed Research* 30:134–41.
- Sherf AF and Macnab AA.1986. *Vegetable Diseases and Their Control*. John Wiley and Sons, New York, USA. 445p.
- Siddaiah CN, Prasanth KVH, Satyanarayana NR, Mudili V, Gupta VK, KalagaturNK, Satyavati T , Dai XF , Chen J, Mocan A, Singh BP and Srivastava RK. 2018. Chitosan nanoparticles having higher degree of acetylation induce resistance against pearl millet downy mildew through nitric oxide generation. *Scientific Reports* 8:2485.
- Singh BM, Singh D and Paul YS.1987. Outbreak of downy mildew of onion in Himachal Pradesh. *Plant Disease Research* 2:124-26.
- Smith RW, Lorbeer JW and Abd-Elerazik AA.1986. Reappearance and control of downy mildew epidemics in New York. *Review of Plant Pathology* 65:106-08
- Song RG, Lu WP, Li CY, Wang J and ShenYJ. 1998. Inheritance of resistance to *Plasmopara viticola* in intraspecific cross of *Vitis amurensis* Rupr. *Acta Horticulturae* 25: 117-22.
- Stevens NE. 1916. A method of studying the humidity relation of fungi in culture. *Phytopathology* 6: 428-32.
- Sticher L, Mauch-Mani B and Métraux JP. 1997. Systemic acquired resistance. *Annual Review of Phytopathology* 35: 235e270.
- Sugha SK and Singh BM. 1991. Status of downy mildew of onion (*Peronospora destructor*) in Kangra district of Himachal Pradesh and its effect on crop yield. *Plant Disease Research* 6:35-38.
- Surviliené E, Valiskaitė A and Raudonis L. 2008. The effect of fungicides on the development of downy mildew of onions. *Zemdirbyste Agriculture* 95: 171-79.

- Tahir M, Mohibullah MS and Saifullah. 1989. The effect of different spray fungicides on downy mildew and yield of onion. *Sarhad Journal of Agricultural sciences* 6: 377-80.
- Takechi Y, Yoki I and Naoto S. 2000. Oligosaccharide elicitors and their receptors in plant defense. *Trends Glycosci. Glycotechnol.* 12:113–20.
- Teviodale BL, May DM, Harper D and Jarde D. 1980. New fungicides apparently control onion mildew. *Review of Plant Pathology* 58:439-41.
- Thines M. 2009. Bridging the gulf: Phytophthora and downy mildews are connected by a rare grass parasite. *Public Library of Science* 4:e4790.
- Timchenko VI. 1979. Disease of onion. *Review of Plant Pathology* 58:439-40.
- Trouvelot S, Varnier AL, Allègre M, Mercier L, Baillieul F, Arnould C, Gianinazzi-Pearson V, Klarzynski O, Joubert J M, Pugin A and Daire X. 2008. A beta-1,3 glucan sulfate induces resistance in grapevine against *Plasmopara viticola* through priming of defense responses, including HR-like cell death. *Molecular Plant-Microbe Interactions* 21: 232–43.
- Van Doorn AM. 1959. Onderzoekingen over het optreden en de bestrijding van valse meeldauw (*Peronospora destructor*) bij uien. *Tijdschrift Over Plantenziekten* 65:193-95.
- Vasyukova NI, Zinoveva SV, Ilinskaya LI, Perekhod CA, Gerasimova NG, Iliana AV, Varlamov VP and Ozeretskovskaya OL. 2001. Modulation of plant resistance to diseases by water-soluble chitosan. *Applied Biochemistry and Microbiology* 37:103–09.
- Vijayaraghavan R, Louis V, Mathew SK, Davis F, Varghese A and Dilna. 2017. *In Planta* studies on downy mildew disease of bittergourd using fungicides and bioagents. *International Journal of Current Microbiology and Applied Sciences* 6: 945-50.
- Vincent JM. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 150: 850-53.
- Viranyi F.1974. Studies on the biology and ecology of onion downy mildew in Hungary I. Overwintering of pathogen in onion. II. Factors influencing sporulation. *Acta Phytopathologica Academiae Scientiarum Hungaricae* 9:311-14.
- Viranyi F. 1975. Studies on the biology and ecology of onion downy mildew (*Peronospora destructor*) Berk. (Fries) in Hungary. Epidemiology of the disease. *Acta Phytopathologica Academiae Scientiarum Hungaricae* 10:321-28.
- Walters D, Walsh D, Newton A and Lyon G. 2005. Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors. *Phytopathology* 95:1368–73.
- Wheeler BEJ. 1972. *An Introduction to Plant Diseases*. John Wiley & sons, London, U.K. 73p.
- Wilson BGI. 1980. Downy mildew control in onion. *Necc, Zeulund Commercial Growerv* 35:27-29.

- Wright PJ, Chynoweth RW, Beresford RM and Henshall WR. 2002. Comparison of strategies for timing protective and curative fungicides for control of onion downy mildew (*Peronospora destructor*) in New Zealand. *Proceeding of BCPC Conference Pests and Diseases*. 1: 207–12.
- Xing K, Zhu X, Peng X and Qin S. 2015. Chitosan antimicrobial and eliciting properties for pest control in agriculture: A review. *Agron. Sustainable Development* 35:569–88.
- Yarwood CE. 1937. Relation of light to the diurnal cycle of sporulation of *Peronospora destructor*. *Journal of Agriculture Research* 54: 365-73.
- Yarwood CE. 1941. Sporulation injury associated with downy mildew infections. *Phytopathology* 51:741-43.
- Yarwood CE. 1943. Onion downy mildew. *Hilgardia* 14:595-601.
- Zaracovitis C.1965. Attempts to identify powdery mildew fungi. *Transactions of British Mycological Society* 48:553-58.

**Dr. YS Parmar University of Horticulture and Forestry**  
**Nauni, Solan-173230 (H.P.)**  
**Department of Plant Pathology**

**Title of Thesis** : “Studies on downy mildew of onion (*Allium cepa* L.)”  
**Name of the student** : Ankita  
**Admission Number** : H-2016-70-M  
**Degree Awarded** : M.Sc. (Plant Pathology)  
**Year of Award of Degree** : 2018  
**Major Advisor** : Dr. Sunita Chandel (Professor)  
**Major Subject/ Discipline** : Plant Pathology  
**Minor Field** : i) Vegetable Science ii) Entomology  
**No. of pages in thesis** : 56 + iii  
**No. of words in Abstract** : 433

**ABSTRACT**

Onion downy mildew disease is a serious threat to Onion production in India. Onion downy mildew is caused by *Peronospora destructor* (Berk.) Casp. ex Berk. which causes heavy losses to onion worldwide as well as in Himachal Pradesh. Therefore detailed investigations were undertaken with the objectives to record the disease severity of downy mildew of onion in Solan district of Himachal Pradesh, and to study the morphological, epidemiological parameters of the pathogen and devise suitable management strategies with SAR inducers and other chemicals. Survey revealed that disease was moderately prevalent in Solan district with severity range varying between 4.78 to 58.65 per cent, maximum at Pandah (58.65 %) and minimum at Dharampur (4.78 %). The pathogen was identified based on the morphological features as *Peronospora destructor* (Berk.) Casp. ex Berk. Most distinguishing morphological features of pathogen were production of hyaline, coenocytic mycelia having width of 3.7-6.8 µm, dichotomously branched sporangiophores and hyaline pyriform/fusiform sporangia measuring in length (50.4-65.8 µm) and width (24.6-25.2 µm). The pathogenicity results were obtained within 8-12 days of inoculation. Among various temperature regimes, the maximum germination (73.96 %) and germ tube length growth (202.98 µm) was observed at temperature 15° C whereas RH levels of more than 95 per cent were suitable for germination as maximum germination (71.4 %) was seen at 100 per cent RH with maximum germ tube length (142.63 µm). Effect of different meteorological parameters on progress of downy mildew of onion (cv. ‘Palam Lohit’) was studied under field conditions and it was observed that the disease severity become apparent in last week of February with minimum disease severity of 3.55% which gradually increase in respective months and assumed highest proportion (72.76%) in the second week of April, 2018. The mean maximum temperature ranging from 21.5-26.6 °C, mean minimum temperature of the range 6.7-17.23 °C, average relative humidity of 52-68%, cumulative rainfall of 1-4.5 mm favoured the disease spread. *In vitro* evaluation of 4 non-systemic and 3 combi-products fungicides revealed that one systemic fungicide Dithane M-45 and two combi-products Ridomil Gold and Cabrio Top were most efficacious. Highest inhibition of sporangial germination (100%) was recorded in Ridomil Gold followed by Dithane M-45 and Cabrio Top while Blitox 50 gave minimum inhibition even at higher concentrations. Best performing fungicides under *in vitro* were tested under *in vivo* conditions along with other three SAR chemicals (Chitosan, Potassium orthophosphate and BABA) and reported their effectiveness by reducing the disease severity to 12.5 per cent in comparison of the control (69.5%) of onion downy mildew and increasing the average plant height, bulb yield to 43.9 cm, 26.7 t/ha, respectively.

**Signature of the Student**  
**Name:** Ankita  
**Date:**

**Signature of Major Advisor**  
**Name:** Dr. Sunita Chandel  
**Date:**

**Countersigned**

**Professor and Head**  
**Department of Plant Pathology**  
**Dr. Y.S. Parmar University of Horticulture & Forestry**  
**Nauni, Solan (H.P.) 173 230**

## APPENDIX-II

### Analysis of variance tables

**ANOVA 1 Effect of different temperature regimes on sporangial germination of *Peronospora destructor* (Table 4.4)**

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Temperature	5	34,914.551	6,982.910	15,123.288
Time	2	115.833	57.917	125.433
Temperature x Time	10	139.065	13.907	30.118
Error	54	24.934	0.462	
Total	71	35,194.383		

**ANOVA 2 Effect of different temperature regimes on germ tube length of *Peronospora destructor* (Table 4.4)**

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Temperature	5	378,025.240	75,605.048	48,791.992
Time	2	1,150.702	575.351	371.305
Temperature x Time	10	1,243.128	124.313	80.226
Error	54	83.675	1.550	
Total	71	380,502.744		

**ANOVA 3 Effect of different relative humidity levels on sporangial germination of *Peronospora destructor* (Table 4.5)**

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Relative humidity	7	41,896.754	5,985.251	35,442.853
Time	2	106.744	53.372	316.053
Relative humidity x Time	14	184.530	13.181	78.052
Error	48	8.106	0.169	
Total	71	42,196.134		

**ANOVA 4 Effect of different relative humidity levels on germ tube length of *Peronospora destructor* (Table 4.5)**

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Relative humidity	7	192,280.406	27,468.629	5,202.596
Time	2	2,749.267	1,374.634	260.357
Relative humidity x Time	14	5,062.399	361.600	68.488
Error	48	253.430	5.280	
Total	71	200,345.502		

**ANOVA 5 Effect of different fungicides on sporangial germination inhibition (%) of *Peronospora destructor* (Table 4.10)**

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Fungicides	6	3,825.120	637.520	5,872.627
Concentration	2	12,257.423	6,128.711	56,455.695
Fungicide x concentration	12	1,774.971	147.914	1,362.538
Time	2	74.230	37.115	341.889
Fungicide x time	12	51.582	4.298	39.596
Concentration x Time	4	26.859	6.715	61.855
Fungicide x concentration x time	24	64.308	2.679	24.683
Error	126	13.678	0.109	
Total	188	18,088.170		

**ANOVA 6 Effect of different fungicides on germ tube length production ( $\mu\text{m}$ ) of *Peronospora destructor* (Table 4.11)**

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Fungicides	6	11,574.504	1,929.084	11,865.798
Concentration	2	32,563.097	16,281.548	100,147.818
Fungicide x concentration	12	4,132.601	344.383	2,118.303
Time	2	413.178	206.589	1,270.729
Fungicide x time	12	61.479	5.123	31.513
Concentration x Time	4	312.368	78.092	480.345
Fungicide x concentration x time	24	53.282	2.220	13.656
Error	126	20.484	0.163	
Total	188	49,130.994		

**ANOVA 7 Effect of SAR and fungicides on onion downy mildew severity (Table 4.12)**

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Replication	2	0.776		
Treatment (SAR + Fungicides)	8	2,917.222	364.653	711.651
Error	16	8.198	0.512	
Total	26	2,926.197		

**ANOVA 8 Effect of SAR and fungicides on plant height (Table 4.12)**

<b>Source of Variation</b>	<b>DF</b>	<b>Sum of Squares</b>	<b>Mean Squares</b>	<b>F-Calculated</b>
Replication	2	1.863		
Treatment (SAR + Fungicides)	8	640.723	80.09	97.677
Error	16	13.119	0.82	
Total	26	655.705		

**ANOVA 9 Effect of SAR and fungicides on bulb yield (Table 4.12)**

<b>Source of Variation</b>	<b>DF</b>	<b>Sum of Squares</b>	<b>Mean Squares</b>	<b>F-Calculated</b>
Replication	2	0.776		
Treatment (SAR + Fungicides)	8	2,917.22	364.653	711.651
Error	16	8.198	0.512	
Total	26	2,926.20		

## BRIEF BIO-DATA

**Name** : **Ankita**  
**Father's Name** : Shri Sunil Kumar  
**Mother's Name** : Smt Anita Kumari  
**Date of Birth** : 26.11.1994  
**Sex** : Female  
**Marital Status** : Unmarried  
**Nationality** : Indian

### Educational Qualifications:

<b>Certificate/ Degree</b>	<b>Month &amp; Year</b>	<b>School/ College</b>	<b>Board/ University</b>	<b>Marks (%)</b>	<b>Division</b>
Matriculation	May, 2009	Kendriya Vidyalaya Muzaffarpur, Bihar	Central Board of Secondary Education (CBSE)	90.4	First
10+2	May, 2011	Kendriya Vidyalaya Muzaffarpur, Bihar	Central Board of Secondary Education (CBSE)	84	First
BSc (Agriculture)	May, 2016	College of Agriculture, Dharwad	University of Agriculture Sciences, Dharwad, Karnataka	87.70	First

Whether sponsored by some State/  
Central Govt/Univ/SAARC : NA

Scholarship/ Stipend/ Fellowship, any  
other financial assistance received  
during the study period : ICAR/NTS scholarship

**Place:** Nauni, Solan (HP)  
**Date:**

**(Ankita)**