Studies on Powdery Mildew (*Erysiphe euonymi-japonici* Veinn-Bourg.) of Euonymus (*Euonymus japonicus* Thunb.) in Kashmir valley

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(2011-363-D)



Division of Plant Pathology

Faculty of Horticulture

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Thesis

Submitted to

Faculty of Horticulture
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In partial fulfillment of requirement for the award of degree of

Doctor of Philosophy in Plant Pathology

2016


Dedicated
to my beloved Parents

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Faculty of Horticulture, Division of Plant Pathology

Certificate - I

This is to certify that the thesis entitled **"Studies on Powdery Mildew (*Erysiphe euonymi-japonici* Veinn-Bourg.) of Euonymus (*Euonymus japonicus* Thunb.) in Kashmir valley"** submitted in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy in Plant Pathology,** to the **Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Mr. Rayees Ahmad Ahanger (Regd. No. 2011-363-D)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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This is to certify that the thesis entitled **"Studies on Powdery Mildew (*Erysiphe euonymi-japonici* Veinn-Bourg.) of Euonymus (*Euonymus japonicus* Thunb.) in Kashmir valley"** Submitted by **Mr. Rayees Ahmad Ahanger (Regd. No. 2011-363-D)** to the Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy in Plant Pathology** was examined and approved by the Advisory Committee and External Examiner on ……

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ABSTRACT

Present investigation "Studies on Powdery Mildew (*Erysiphe euonymi-japonici* Veinn-Bourg.) of Euonymus (*Euonymus japonicus* Thunb.) in Kashmir valley" revealed that the disease was prevalent in all the zones of Kashmir valley surveyed during 2012 and 2013. The average disease incidence of 75.14 and 77.60 per cent on leaves and 21.23 and 22.74 on twigs with per cent disease intensity (PDI) of 42.60 and 45.05 on leaves and 11.79 and 12.90 on twigs was recorded during the two years, respectively. The overall disease incidence ranged from 56.67 to 86.70 per cent on leaves and 8.19 to 31.27 on twigs and PDI varied from 20.89 to 53.33 on leaves and 3.90 to 19.20 on twigs. The highest average disease incidence and PDI of 78.06 and 48.01 per cent on leaves and 23.59 and 13.31 per cent on twigs was recorded in south zone whereas, least disease incidence (73.83%) and PDI (40.02%) on leaves (73.83 and 40.02%) and twigs (19.16 and 10.54%) was recorded in north zone. Among the locations, Nishat exhibited maximum disease incidence (86.70%) and PDI (53.33%) on leaves as well as on twigs (31.27 and 18.80%), whereas, Iqbal Park exhibited the minimum disease incidence (56.67%) and PDI (20.89%) on leaves and twigs (8.19 and 3.62%). The powdery mildew was noticed to be characterized by the appearance of chlorotic spots on upper leaf surface with whitish powdery growth on corresponding lower

surface. The disease ultimately resulted in longitudinal folding, curling and distortion of leaves, which finally got shriveled and withered. The pathogen *Oidium euonymi-japonici* (anamorph) overwintered asexually as mycelium on intact infected twigs which started producing conidia in the 4th week of February and continued till ending October. The pathogen ceased to produce conidia from the 1st week of November to 3rd week of February, 2012-2013 and 2013-2014. On intact-overwintered infected twigs, maximum conidial production and viability was observed during 1st week of April and 1st week of August in both the years. However, no conidial formation was observed on the twigs, kept 10 cm deep in the soil. The mycelium was branched, septate and hyaline. Conidia were elliptical and hyaline produced on unbranched and hyaline conidiophores. Epidemiological studies further revealed that maximum and minimum temperature exhibited significant positive correlation with the PDI. The minimum relative humidity exhibited significant negative correlation with the PDI, whereas maximum relative humidity and rainfall showed non-significant negative correlation. *In vivo* evaluation of different test fungicides revealed that flusilazole 40 EC (@ 0.02%) was significantly superior in controlling the disease both on leaves and twigs, while as potassium bicarbonate ($KHCO_3$) was found to be least effective. *In vivo* evaluation of bioagents revealed that *Ampelomyces quisqualis* was significantly superior followed by *Pseudomonas fluorescens* (both at 50% concentration) in reducing the PDI whereas, *Trichoderma viride* proved to be least effective. Pruning of twigs 6 cm below diseased area proved significantly superior to other pruning levels in checking the disease. Out of 6 cultivars screened, Silver King and Silver Queen were found to be resistant andPatens as susceptible whereas, Emerald Gaiety, Canadale Gold and Box-leaf Euonymus proved to be moderately resistant in their reactions to the powdery mildew. The present investigation revealed that alternate sprays of flusilazole (@ 0.02%) and *A. quisqualis* (@ 50%) alongwith pruning of twigs (6 cm below diseased area) proved to be most effective integrated disease management strategy, by exhibiting 2.33 and 0.16 PDI on leaves and twigs compared to 56.40 and 17.15 recorded in check, respectively.

Keywords: *Ampelomyces quisqualis, Erysiphe euonymi-japonici, fungitoxicant, Oidium euonymi-japonici*, Powdery mildew, Euonymus

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Rayees Ahmad Ahanger

Place: Shalimar, Srinagar.
Dated:

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Chapter-1

INTRODUCTION

Euonymus japonicus Thunb., also known as spindle tree, belonging to Celastraceae family is a popular ornamental plant in its native areas Japan, Korea, China, Europe, North America and other regions of the world. *E. japonicus* is a branched evergreen shrub with green and smooth twigs having lightly serrated, thick, dark green and waxy leaves (Orwa *et al*., 2009). Euonymus is popularly recognized for its colourful and variegated foliage that differs greatly from one cultivar to another (Randolph *et al*., 1981). The genus *Euonymus* contains approximately 200 species worldwide, ranging from evergreen shrubs, such as *E. japonicus*, to evergreen vines, such as *E. fortune* (Wintercreeper), to deciduous groundcovers, such as *E. obovata* (Garbary and Deveau, 2007). All euonymus species prefer full sun, fertile, moist and well-drained soil but do not tolerate waterlogged soil.

The euonymus is used as a decorative garden plant, specimen foundation plant and natural or sheared hedge or screen in landscaping (Ningen *et al*., 2005). Some species of euonymus (e.g. *E. alatus)* contain a glycoside kaempferol and quercitin which reportedly reduce the risk of developing several diseases such as cancer and cardiovascular diseases, and possess antioxidant, anti-inflammatory, antimicrobial, cardioprotective, neuroprotective, anti-diabetic, anti-osteoporotic, analgesic and anti-allergic activities (Fang, 2008; Wang *et al*., 2009; Montano *et al*., 2011).

E. japonicus has remained a dominant landscape element in the form of hedges and screens in most of the public and private gardens in Kashmir valley. Its evergreen nature, fine texture, thick lustrous leaves and ability to withstand rigorous pruning makes it an ideal choice for use as a hedge and screen plant in various landscape situations. Different species of euonymus in general and *E. japonicus* in particular are grown in almost all the floriculture gardens and

private lawns for aesthetic ornamental value throughout the Kashmir valley. Thereby, signifying its commercial importance for floriculture-nursery industry.

About ten thousand plant species are known to be hosts of powdery mildews, including different ornamental plants like euonymus, azaleas, catalpa, cherry, crab apple, privet, rose, rhododendron, dogwood, honeysuckle, lilac, silver maple and willow (Timmerman, 2010). Due to their epidemic potential and wide occurrence, powdery mildews are of high economic relevance on important crops (Brand, 2009).

E. japonicus is prone to various diseases but is highly susceptible to powdery mildew throughout world including Kashmir valley (Koul, 1980). Powdery mildew appears as white to grey colonies on leaves and twigs. The disease severely downgrades the aesthetic appearance of the foliage and appears to be the main cause for the decline of Euonymus in Kashmir. Powdery mildew of *E. japonicus* was first reported from Italy in 1900 by Acrangli, and later on in 1903, from Austria and England by Salmon (Braun *et al*., 2002). In Kashmir, the disease was first reported in 1980 (Koul, 1980).

The fungus causing powdery mildew was first described by Saccardo in 1903 as *Oidium euonymi-japonici*. The first report of cleistocarps was given by Viennot-Bourgin (1965). The fungus *O. euonymi-japonici* overwinters as mycelia and spores on infected leaves and stems and the infection is favoured by temperatures of 25 to 32^{o}C and relative humidity between 85 and 100 per cent (Van, 2008). Free water on leaf surfaces reportedly suppressed spore germination (Pscheidt and Ocamb, 2015).

The use of fungitoxicants still appears to be necessary to control various diseases of euonymus, though their use could be reduced or even avoided on the cultivars with high level of resistance to powdery mildew (Hansen, 2009). Different chemicals have been tested for management of powdery mildew of euonymus world over which include myclobutanil, triadimefon, azoxystrobin,

propiconazole, hexaconazole, dinocap, sulphur etc. (Alan, 1998; Dhruj *et al*., 2000; Moyer and Peres, 2012; Williamson, 2013), besides foliar application of various polymer coatings or aquous solution of sodium or potassium bicarbonate along with horticultural oil (Ziv and Hagiladi, 1993).

The use of chemicals raises concerns about the environment and human health hazards (Rankovic, 1997). Therefore, the importance of biological methods for plant protection has increased today. In most cases, planting resistant varieties or avoiding the most susceptible varieties adopting following good cultural practices has been advocated for control of plant diseases including powdery mildew of *E. japonicus* (Olsen, 1998; Hagan and Mullen, 2000). The use of hyperparasite fungi and other biocontrol agents effectively tested against the pathogen include *Ampelomyces quisqualis*, *Cladosporium* spp, *Trichothecium* spp, *Trichoderma* spp, *Bacillus subtilis* and *Penicillium chrysogenum* (Hijwegen, 1988; Elad, 2000; Beckerman, 2008; Moyer and Peres, 2012). Amongst them, *A. quisqualis* has been reportedly observed to be widely distributed on the powdery mildews and considered as hyperparasite of the pathogen (Rankovic, 1997). Pruning of heavily diseased branches, planting in sunny area and avoiding crowding have also been advocated as management strategies for the disease (Williamson, 2013).

Most of the euonymus hedges in Kashmir gardens have succumbed to powdery mildew, a disease that makes the plant unsightly so much so that even old and established hedges have been uprooted. Garden lovers are reluctant to create new euonymus hedges both in private and public landscape spaces. There is a need to study the fungus and develop a management strategy to restore euonymus as a dominant hedge plant in valley gardens. However, no study has been conducted in Kashmir on various aspects of the disease so far. Therefore, the present study on powdery mildew of *E. japonicus* was carried out with the following objectives:

- To study the status of the disease.
- To study symptomatology, perpetuation and epidemiology of the disease.
- To screen the available germlasm against the pathogen.
- To devise strategy/strategies for the management of disease.

Chapter-2

REVIEW OF LITERATURE

The ability of the powdery mildew fungi to coexist on their evergreen ornamental hosts is obviously advantageous to them in view of the obligate nature of their parasitism. *Euonymus japonicus* Thunb., commonly known as Japanese spindle tree or evergreen tree, is a popular, woody, ornamental plant often grown in parks and gardens, especially to form hedges and even grows wild in some areas. The leaves are extremely dark or glossy green. Powdery mildew, caused by *Oidium euonymi-japonici*, has been a major disease problem on euonymus. Symptoms appear as flat white to grey powdery areas on foliage, particularly on young foliage and buds (Ellis and Ellis, 1997).

2.1 Occurrence

Powdery mildews are caused by approximately 1,100 species of fungi belonging to six closely related genera viz., *Erysiphe*, *Microsphaera*, *Phyllactinia*, *Podosphaera*, *Sphaerotheca* and *Uncinula*, and the genus *Oidium* represents their imperfect state (Pataky, 1987). Nearly 700 species of powdery mildew fungi have been reportedly occurring on about 7600 species of angiosperms including ornamentals (Braun *et al.*, 2002).

While stating the occurrence of powdery mildew on many herbaceous and woody ornamentals under greenhouses, nurseries or landscape settings, Windham (1996) reported that powdery mildews caused serious damage to woody ornamentals such as euonymus, rose, flowering dogwood, crape myrtle, common lilac, Persian lilac, besides herbaceous ornamentals such as African violet, begonia, chrysanthemum and kalanchoe and bedding plants such as zinnia and snapdragon.

Braun *et al.* (2002) studied that there were nearly 700 species of powdery mildews, occurring on about 7600 species of angiosperms including ornamentals. Infection can damage tissue or whole plants, causing defoliation, cosmetic

damage and reducing yields, quality, aesthetic and decorative values of ornamentals like euonymus, dogwood and crape myrtle.

2.2 Morphology

Viennot-Bourgin (1965) reported that the conidia of *O. euonymi-japonici* were 21-36 × 7-13 µm in size. Salmon (1905) studied that the conidia of the fungus *O. euonymi-japonici* were 30-38×13-14 µm in size.

Braun *et al*. (2002) reported that despite of morphological variations, the genus *Oidium* was relatively homogenous and all states of *Oidium* belonged to powdery mildews (Erysiphaceae).

Braun *et al*. (2002) furthur reported that the primary mycelium of powdery mildews was hyaline, septate, thin walled with uninucleate and vacuolated hyphal cells with width varying from 10 to 20 µm and the length between 20 and 100 µm. The hyphae were branched and ramify at more or less right angles.

Li *et al.* (2011) reported that the fungus *Erysiphae euonymi-japonici* causing powdery mildew of *E. japonicus* had unbranched conidiophores with an average length of 57.3 µm and width of 8.3 µm. Cylindrical to ellipsoid-cylindrical conidia with an average length of 27.7 µm and width of 10.4 µm were borne singly.

2.3 Symptomatology

While studying the symptoms of powdery mildew on five ornamentals viz., lisianthus (*Eustoma grandiflorum*), nasturtium (*Tropaeolum majus*), lily (*Zantedeschia aethiopica*), impatiens (*Impatiens balsamin*a) and balloon plant (*Asclepias physocarpa*), Reis *et al*. (2007) reported that the typical symptoms of the disease appeared as chlorotic areas on the upper leaf surface corresponding to a fungal colony in the lower leaf surface and these chlorotic areas eventually turned into brown necrotic lesions.

Mameth and Chatfield (2009) reported that powdery mildews of the

landscape ornamentals like euonymus, dogwood and crape myrtle often appeared as a superficial white or gray powdery growth of fungus over the surface of leaves, stems, flowers or fruits. These patches enlarged until they covered the entire leaf on one or both sides. Young foliage and shoots were most susceptible. Leaf curling and twisting was noted and severe powdery mildew infection resulted in yellowed, dried and brown leaves with disfigured shoots and flowers.

Powdery mildew disease, caused by different species of fungi, affects a wide range of ornamentals and is considered to be easy to diagnose since its symptoms are usually quite distinctive (Severoglu and Ozyigit, 2012). Li *et al*. (2011) observed that on severely infected leaves of euonymus, mycelia were amphigenous, thick, forming irregular white patches, and effused to cover the whole surface. Subsequently, these leaves became chlorotic, curled, and withered.

Severoglu and Ozyigit (2012) reported that powdery mildew usually produced a white powdery substance that grew on both surfaces of leaf and infected leaves became twisted, distorted and wilted. They reported that spores of all powdery mildew causing species could germinate and cause infection in the absence of water, though humidity requirements for spore germination varied.

Williamson (2013) reported that powdery mildew of euonymus was the most common and the most difficult disease to control and caused yellowing of leaves and severe infections led to slight leaf drop, but heavy leaf drop was not a characteristic symptom of the disease. On young shoots, the leaves became curled.

2.4 Perpetuation

Salmon (1905) observed that fungus *Oidium euonymi-japonici* overwinters as mycelia on leaves, whereas Horst (1990) reported its overwintering as mycelia and spores both on infected leaves and stems, and the airborne spores spread to newly emerging leaves in the spring to cause fresh infection. Wheeler (1978) reported that the infection of leaves results in the formation of suberized

layer in which mycelium and chlamydospores hibernated and caused infection of new growth in the following spring.

On other crops, the powdery mildew causing fungi reportedly perpetuate as mycelia on bud scales as well as ascospores in cleistothecia as in case of powdery mildew of grape vine (Weltzien and Weltzien, 1962). However, Sall and Wrysinski (1982) reported that early infections on grapes in the spring aroused from the mycelia in buds and ascospores were of minor importance for the epidemiology of *Oidium.* In case of powdery mildew of mango, Gupta (1989) reported that causal fungus *Oidium mangiferae* perpetuated in the form of mycelium and conidia or dormant mycelium on older infected mango leaves and intact green malformed inflorescences.

Wheeler (1978) stated that in Japan the powdery mildew on *E. japonicus* was named *Uncinula euonymi-japonicae* by Hara in 1921 but this stands unclear whether it was based on a collection of the perfect state as other workers failed to find any cleistocarp. However, the first record of formation of cleistocarps of Microsphaera type was reported by Viennot-Bourgin (1965). Later on, Stephen (2010) also reported that the fungus could form tiny black fruiting bodies (cleistothecia) within the white mycelium on severely infected and prematurely fallen leaves.

In case of powdery mildew of rose, Horst (1983) reported that the causal fungus *Sphaerothica pannosa* overwintered as mycelium or conidia or ascospores produced in fallen leaves, but new shoots were infected by air borne conidia in spring and summer months, whereas Foschi *et al*. (1993) noticed that the main form of overwintering was through mycelium on rose buds and flowers.

Olsen (1998) reported that powdery mildew fungi survived in low deserts as short-lived windborne spores and as mycelium in the host plant tissue. In colder areas where freezing temperatures are common throughout the winter months, powdery mildew fungi survived as mycelium in the host tissue and by

forming a sexual stage with spores that are resistant to drying and cold temperatures. The sexual spores germinate in the spring to start the disease cycle over again.

Fraedrich (1999) reported that approximately six genera of fungi caused powdery mildew on specific ornamentals. The fungi overwintered as mycelium in the buds or as spores on fallen leaves.

Shi and Mmbaga (2006) reported that *Erysiphe lagerstroemiae* and *Erysiphe australiana* causing powdery mildew of crape myrtle overwintered as mycelia on dormant buds. They also observed that the teleomorph rarely developed in crape myrtle, and ascocarps were never formed. Some powdery mildew fungi, such as *E. cichoracearum* may develop cleistothecia with ascospores by the end of the season (Braun *et al*., 2002).

Van (2008) reported that *O. euonymi-japonici* survived from season to season on infected tissues such as leaves and stems and the spores of the powdery mildew fungi were often carried by wind currents or splashing water.

2.5 Host range

Salmon (1905) reported that *Euonymus europaeus* cannot be infected with conidia from *Erysiphe euonymi japonici.*

Wheeler (1978) reported that powdery mildew on the related *Euonymus europaeus* appeared to be a distinct species of *Microsphaera euonymi*, a fungus originally described by Decandolle as *Erysiphe euonymi* in 1815.

Johnson *et al*. (1984) reported that powdery mildews had more difficult germinating and producing infection structures on non-hosts than on hosts.

Dreistadt *et al*. (1994) studied that *O. euonymi-japonici* infected the other species of Euonymus like *E. albomarginatus, E. aureomarginatus, E. aureopictus* and *E. microphyllus.*

Khodaparast and Abbasi (2009) presented comprehensive review of the

species, host ranges and geographical distribution of powdery mildew fungi in Iran and reported that the powdery mildew fungi belonged to eight teleomorphic genera and one anamorphic genus, *Oidium* (five species). The check list of the different reviewers presented by Khodaparast and Abbasi in Iran showed that *O. euonymi-japonici* (Arcang.) Sacc. occurred on *E. japonicus* throughout the country.

Severoglu and Ozyigit (2012) collected the diseased samples of powdery mildew of different plants from urban lands, especially in parks, public and private gardens, road edges, medians and nurseries in 36 of 39 districts of Istanbul. They observed 43 powdery mildew fungal species on 73 different plant species. Some of these fungi observed on a single plant species were *Erysiphe convolvuli* var. *convolvuli*, *E. flexuosa*, *E. lagerstroemiae*, *E. platani*, *E. sparsa*, *E. syringae*, *E. trina*, *E. viburni*, *Leveillula taurica*, *Microsphaera alni* var. *vaccinii*, *M. alphitoides*, *M. berberidicola*, *M. diffusa*, *M. grossulariae*, *M. platani*, *M. quercina*, *Oidium* sp., *O. begonia*, *O. euonymi-japonici*, *O. lauraceum*, *O. lini*, *Peronospora erodii*, *Phillactinia guttata*, *Sphaerotheca fusca*, *S. humuli*, *S. lanestris*, *S. macularis*, *S. pannosa* var. *rosae*, *Uncinila aceris*, *U. circinata*, *U. clintonii*, *U. geniculata*, *U. necator*, *U. Parvula* and *U. salicis*. Some powdery mildew fungi were cosmopolitan and showed parasitic features for many different plant species. These fungi were *Microsphaera alni* (11 plant species), *Erysiphe polygoni* (9 plant species), *Erysiphe cichoracearum* and *Phyllactinia corylea* (8 plant species).

2.6 Epidemiology

Yarwood *et al*. (1954) considered that among plant pathogens the powdery mildews had low optimum temperature range averaging 21^{o}C.

Van der Plank (1963) studied the rate of disease development per unit per day and concluded that powdery mildews and rusts were compound interest diseases, which complete many generations on the crop in a season. Initially

disease starts very slowly, builds up the inoculum in Logarithmic phase. Often nearly half the amount of time needed for an epidemic is spent in logarithmic phase itself. From around 0.5 or 1 per cent severity disease growth was very rapid and multiplication is at an exponential rate.

Another factor attributing to epidemic development was susceptibility of host chiefly in relation to physiological factors, such as host age and susceptibility at an early age, although microclimatic factors due to greater density of foliage in older crops might also be of some importance for infection (Palti, 1971).

Reuveni and Rotem (1973) reported that the relatively low humidity of 55-60 per cent improved sporulation of *S. fuliginea* on squash.

Dixon (1978) reported that, the conidia of *Leveillula taurica* germinate best at 26^{o}C on a dry slide with 52-75 per cent relative humidity.

Koul (1980) reported that powdery mildew of *E. japonicus* was severely destructive from early spring until early autumn in Kashmir.

Gupta (1989) reported that powdery mildew of mango occurred severely at temperature 26°C and 100 per cent relative humidity. However, the fungus could cause infection at 15 to 32°C and 15.5 to 100 per cent relative humidity. He also observed that atmospheric temperature was most important factor for onset and epidemic development of the disease under field conditions. The temperature range of 11-14°C (minimum) and 17-31°C (maximum) along with moderate relative humidity (64-72%) was most congenial for the development of powdery mildew of mango. Rainfall did not play any significant role on appearance and disease development, but dry weather favoured the development of the disease.

Singh *et al*. (1991) developed regression equation indicating a linear relationship between powdery mildew severity and grain yield of mung bean.

Windham (1996) reported that powdery mildew on ornamentals like flowering dogwood (*Cornus florida*), crape myrtle (*Lagerstroemia indica*), euonymus (*Euonymus* spp.) and rose (*Rosa* spp.) produced airborne spores and

usually infected plants when temperatures were moderate (18-32^{o}C) and temperatures above 32^{o}C inhibited growth of the fungus.

Similarly, a crop loss model in the form of Y= 3.49 - 0.558 (AUDPC) with coefficient of determination of 96 per cent was developed to estimate yield loss due to sunflower rust (Patil, 1996).

Olsen (1998) reported that powdery mildews of ornamentals were favoured by moderate to high humidity and low light intensity whereas, at higher elevations, powdery mildew fungi appeared when the host plant begin to grow new foliage in the spring and continued to infect plants until late fall.

Solanki *et al*. (1999) used correlation analysis and multiple linear regression and stepwise multiple regression analysis to identify the relationship between severity of powdery mildew of chilli and weather parameters and they reported that, mean temperature, number of trapped conidia, crop age and cumulative number of trapped conidia were highly correlated with the disease.

Upasana and Munshi (2002) studied the development of powdery mildew of wheat in relation to environmental conditions. Disease development was significantly correlated with maximum and minimum temperature at Ludhiana under both the field and glasshouse conditions. Correlation coefficients were highly positive and significant between temperature and per cent disease index.

Carroll and Wilcox (2003) reported that optimum 85 per cent relative humidity was required for the development of grapevine powdery mildew. They concluded that humidity played a significant role in grapevine powdery mildew epidemiology.

Sharma (2003) studied that powdery mildew fungi *like O. euonymi-japonici* and *Sphaerothica pannosa* were favored by high humidity in the plant canopy and warm temperatures (25^{o}C) and fungal spores could not germinate in free water.

Sinha (2005) carried out an experiment at New Delhi to study the effect of temperature and hours of high relative humidity on powdery mildew infection caused by *Oidium erysiphoides* in jujube (*Ziziphus mauritiana* L.). Infection index on fruits was highest (0.96) at temperature 24-26^{o}C, while lower index (0.1- 0.15) was at temperature 10 and 34^{o}C. He also reported that Infection index increased from 0.17 to 0.90 with the increase in hours of relative humidity from 5 to 24 per cent.

Jacob *et al*. (2007) while studying *Oidium neolycopersici* causing powdery mildew of tomato found that severity of powdery mildew was positively correlated with the temperature range from 15 to 25°C, and relative humidity of 50 to 60 per cent. Conversely, disease was negatively correlated with the duration of temperatures in the low and high ranges (5 to 15°C and 35 to 40°C), with the duration of relative humidity levels of 40 per cent and below, and with 50 to 60 per cent relative humidity. High relative humidity of 90 to 100 per cent was also negatively correlated with disease severity.

Jat and Goyal (2009) evaluated that temperature had significant and humidity had non significant correlation with per cent disease intensity of powdery mildew of ber incited by *O. erysiphoides* f. sp. *ziziphi*. Matrix studies indicated that there was a significant relation between PDI and temperature.

Xun (2009) observed that the occurrence of powdery mildew on *E. japonicus* had obvious seasonality with 2 peak periods of disease from middle of April to middle of May and from early September to early October.

2.7 Chemical management

Keeping in view, the serious damage caused by the powdery mildew pathogen *E. japonicus*, various workers had stressed the need for chemical treatment to the disease (Nademejad, 1966; Orwa *et al*., 2009). Elemental sulphur was one of the first fungicides to be introduced to prevent plant diseases including powdery mildew infections (Severoglu and Ozyigit, 2012). Sulphur products have

been used to manage powdery mildews for centuries but are reportedly effective only when applied before the appearance of disease (Gublar, 2009).

The fungitoxicants that proved effective for the management of powdery mildews of other crops include dinocap or sulphur for inhibiting spore germination of the pathogen *Oidiopsis taurica* (Telomorph =*Leveillula taurica*) and its disease control on chilli (Maharshi and Siradhana, 1984; Saroj and Satish, 1985); carbendazim (@ 0.1%) and penconazole (@ 0.1%) for inhibiting conidial germination of *O. erysiphoides* f. sp. z*iziphi*, infecting ber (Sataraddi,1994); penconazole (@ 0.1%), difenconazole (@ 0.1%), carbendazim (@ 0.1%) and wettable sulphur (@ 0.3%) for inhibiting conidial germination of *E. polygoni*, infecting green gram (Kunkalikar, 1989; Venkatrao, 1997); triadimefon (@ 0.05%) on pea (Gupta and Shyam, 1998); dinocap on coriander, coupled withits early planting (Kalra *et al*., 2000); propiconazole, penconazole, hexaconazole, triadimefon, tridemorph, dinocap and sulphur against *Leveillula taurica*on fenugreek (Dhruj *et al*., 2000); sodium bicarbonate and potassium bicarbonate for inhibiting conidial germination and infection of tomato (Hseih *et al.,* 2005); in case of *S. pannosa* on roses, dinocap or Folicur 50 WG (@ 0.1%) sprayed twice at 14-day interval or 4 times at 7-day interval (Mathur *et al*., 1991; Wojdya and Jaworska, 2005) or difenconazole (@ 0.05%) sprayed at 8-day interval (Kakade *et al*., 2006).

Ziv and Hagiladi (1993) observed that foliar application of various polymer coatings or an aqueous solution of sodium or potassium bicarbonate along with horticultural oil to plant foliage effectively controlled powdery mildew on *E. japonicus*.

While evaluating various commercial formulations of fungitoxicants, Windham (1996) observed that Bayleton 25 WP, Cleary 3336-F, Funginex 6.5EC, Rubigan AS , Strike 25 WP and Immunex controlled powdery mildew of different ornamentals like euonymus, flowering dogwoods, and zinnia.

Dhruj *et al*. (2000) reported that of the seven fungicides viz., propiconazole, penconazole, hexaconazole, triadimefon, tridemorph, dinocap and sulphur, used to control powdery mildew (*Leveillula taurica*) of fenugreek significantly reduced the disease as compared to the control.

Hagan and Mullen (2000) reported that azoxystrobin, fenarimol, myclobutanil, propiconazole, triadimefon, thiophanate methyl, mancozeb, chlorothalonil provided excellent control of powdery mildews.

Dik *et al*. (2003) recommended the use of bicarbonates as a good alternative for the control of powdery mildew of tomato (*Oidium lycopersicum*), sweet pepper (*L. taurica*) and rose (*S. pannosa*) under glasshouse conditions.

Zhang *et al*. (2003) evaluated the efficacy of mencozeb, triademefon, carbendazim, sulphur, tuzet and chlorothalonail against powdery mildew (*S. pannosa*) on rose and showed that mancozeb and sulphur were most effective resulting in 100 per cent control of powdery mildew.

Rajapakse *et al.* (2006) evaluated wettable sulphur @ 5g/l, Neemarin 15% @ 3ml/l, Neem seed extract @5g/l, Caster seed extract @ 5g/l, against *Oidium* spp. causing powdery mildew of rambutan in Sri Lanka. The results showed that spraying of wettable sulphur (5g/l.) at young foliage stage, at flowering, just after fruit setting and 3 weeks after fruit setting on to the plants completely controlled mildew disease of rambutan.

While evaluating various fungicides against powdery mildew pathogen of *E. japonicus*, Qilil *et al*. (2007) found that chlorthalonil inhibited spore germination by 99.3 per cent under *in vitro* conditions and recorded 85 per cent disease control in the field. Van (2008) also observed that foliar application of sulphur, propiconazole, triadimefon and myclobutanil provided excellent disease control in euonymus, crape myrtle and dogwood.

Hartman (2008) reported that the powdery mildew of ornamentals and valuable susceptible trees in the landscape was controlled by applications of triadimefon, myclobutanil, propiconazole and potassium bicarbonate.

Williamson (2013) noticed that fungicides myclobutanil, propiconazole, and thiophanate-methyl protected foliage of euonymus from the infection of powdery mildew by killing the mildew spores as they germinate.

El-Mougy *et al.* (2014) while evaluating the efficacy of different plant resistance inducers and bio-agents against foliar diseases incidence revealed that plant spray with potassium bicarbonate + Thyme resulted in the significant reduction in powdery mildew incidence and severity on cantaloupe plants carried out under commercial plastic houses conditions.

2.8 Biological control

Yarwood (1932) observed killing of clover powdery mildew fungus in experimental plots of red clover by *A. quisqualis.* The first report of *A. quisqualis* on *Oidium* sp. parasitising, *Phaseolus vulgaris* was made by Sydone and Butler from Pusa, Bihar (Chona and Munjal, 1956).

Nagaraj (1966) in his work observed that the *Oidium* sp. on *Striga lutea* being parasitised by *Ampelomyces* sp. and species of Phoma and Alternaria.

Gu and Ko (1997) reported that the most important hyperparasite for powdery mildew of *E. japonicus* was *A. quisqualis.* The mycoparasite *Ampelomyces quisqualis* and *Bacillus subtilis* provided excellent control of powdery mildew of melon seedlings grown under green house conditions (Romero *et al*., 2007).

Ravikumar (1998) found that, different biocontrol agents *viz*. *Trichoderma harzianum* Rifai., *T. viride* Pers., *P. fluorescens* Migula and *Gliocladium virens* Millar. were less effective against powdery mildew of rose, both in laboratory and field conditions compared with fungicides and plant products.

Biju (2000) noticed that the biocontrol agents *T. harzianum* (0.4%), *T. viride* (0.4%) and *P. fluorescens* (0.2%) tested against powdery mildew of peas were less effective, both under laboratory and field conditions as compared to plant products like nimbicidin (0.4%) and ovis (0.15%)

Romero *et al.* (2007) reported that the most effective biocontrol agent of powdery mildew in cucumber was a mycoparasite, *A. quisqualis*.

Elwenees-Elkot and Hamed-Derbalah (2011) evaluated culture filtrates of certain microbial isolates of *Epicoccum nigrum*, *Epicoccum minitans*, *Epicoccum* sp., *T. harzianum*, *T. viride* and *Bacillus pumilus* alone and in combination. The results showed that culture filtrates of different microbial isolates except for *T. harzianum* were more effective against powdery mildew in squash than the tested fungicide alone at the recommended levels. The results also showed that mixing different culture filtrates with penconazole improved efficiency against powdery mildew compared to using the fungicide alone, in both tested seasons. The efficacy of the culture filtrates of the tested microbial isolates against powdery mildew were due to the presence of a mixture of known antifungal compounds.

Pawar (2015) evaluated *T. viride, A. quisqualis, Cladosporium cladosporioides, Fusarium pallidoroseum, Aspergillus niger, Alternaria alternata,* and *Pestalotiopsis dissminata* against *Sphaerotheca fuliginea* (Schlecht ex. Fr.) causing powdery mildew of pumpkin (*Cucurbita maxima*) and identified *A. quisqualis* significantly effective than *F. pallidoroseum, C. cladosporioides* in disease control. Disease control was better in pre-inoculation spray application of biological control agent as compared to post inoculation application.

2.9 Cultural management

Heimann (1996) reported that powdery mildew of ornamentals occurred in severe form on young and succulent growth and the disease could be reduced by lowering nitrogen and increasing potassium fertilization.

Fraedrich (1999) reported that the incidence and severity of powdery mildew of ornamentals like crape myrtle and euonymus could be reduced by planting the ornamentals at least partial sun and by pruning periodically to allow proper light and air penetration. Fertilization in the dormant season would prevent excessive late summer growth, which was most susceptible to damage by powdery mildew. Good sanitation practices through which infected leaves were gathered and destroyed in the autumn would reduce the amount of inoculum available the following year.

Olsen (1998) observed that keeping plants well spaced, properly thinned, consistent application of water, planting susceptible plants in sunny locations and pruning of diseased portions reduced the powdery mildew infection of euonymus.

La-Mondia (2001) reported that propagation of diseased cuttings or cuttings with latent infections favoured selection for fungicide resistance in a particular isolate over several generations of plants.

Van (2008) reported that powdery mildew of ornamentals like euonymus dogwood and crapemyrtle could be minimized by reducing leaf wetness. Susceptible plants grown and maintained in well-spaced, sunny locations had a lower incidence of powdery mildew than those grown in shady, humid conditions. He further reported that the fallen leaves should be removed and destroyed during the fall or winter since they were important sources for infections in the spring.

Hansen (2009) reported that cultural practices that reduce humidity, such as pruning for improved air circulation and avoiding shady locations, helped prevent powdery mildew of ornamentals.

2.10 Screening

Dekker (1976) reported that chemicals often failed to control the powdery mildews because the pathogen developed resistance.

Hagan and Mullen (2000) reported that the variegated cultivars of euonymus (*E. japonicus*) were more resistant to powdery mildew than green and

non-variegated varieties.The green cultivar Patens is very susceptible.

Van (2008) studied that the best control for powdery mildew of ornamentals like euonymus, dogwood and crape myrtle was to plant resistant varieties.

In the past, the fungus was rarely observed on variegated cultivars but now it was much more common (Pscheidt and Ocamb, 2015). Dreistadt *et al*. (1994) also reported that the variegated varieties of euonymus like aureomarginatus, albomarginatus, aureopictus were more resistant than non-variegated types like patens

2.11 Integrated disease management

Casey *et al*. (2007) launched an integrated pest management programme with the aim to reduce the risk of pesticides and to utilize the biological control agents with cultural management practices to manage the key pests of floriculture crops.

Hong (2014) developed integrated disease management strategy for ornamentals and reported that it was important to remove dead and diseased plant parts as well as to keep the tools clean for the effective control of powdery mildew of ornamentals. Removing dead plants reduced inoculum during a growing season and removing diseased plant parts avoided unintentional spreading of a pathogen from one plant to another. Symptomatic twigs, leaves and flowers should be pruned from plants and removed from the bed. Sterilize pruners with 70 per cent alcohol solution should be done regularlys. He also reported that gardening exclusively with resistant plant species and cultivars was an option for gardeners who want to avoid and manage disease problems and when chemical control was necessary, it was critical to first determine the nature of a disease, and then select the right chemical. It was best to alternate pesticides with different modes of action.

Chapter - 3

MATERIALS AND METHODS

The present study was carried out in the Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar, Srinagar. The field trials were laid out at Shalimar campus and the pot trials were laid out in the Division of Plant Pathology, SKUAST-K. Three zones of Kashmir valley *viz.*, central, south and north were surveyed to ascertain the status of the disease. The details of materials used and the methodology followed during the course of this investigation are explained here under.

3.1 Disease status

Extensive surveys were carried out in central, south and north zones of Kashmir valley during the peak period of the disease (4^{th} week of June), in the year 2012 and 2013, for recording disease incidence and per cent disease intensity (PDI) of powdery mildew of euonymus. In each zone four locations were selected with three sites per location and ten plants per site. Per cent disease incidence was calculated by counting the total number of leaves and the number of twigs infected on randomly selected plants using the formula:

$$\text{Per cent disease incidence} = \frac{\text{No of diseased leaves/twigs}}{\text{Total No. of leaves/twigs}} \times 100$$

The per cent disease intensity (PDI) on leaves and twigs was assessed by using 0-5 rating scale (Plate 1&2) as given by Mirza (1999). Six disease categories were made on the basis of per cent leaf area infected as per the following key:



Plate-1: **Assessment scale for disease intensity of powdery mildew on leaves of *Euonymus japonicus* caused by *Oidium euonymi-japonici***

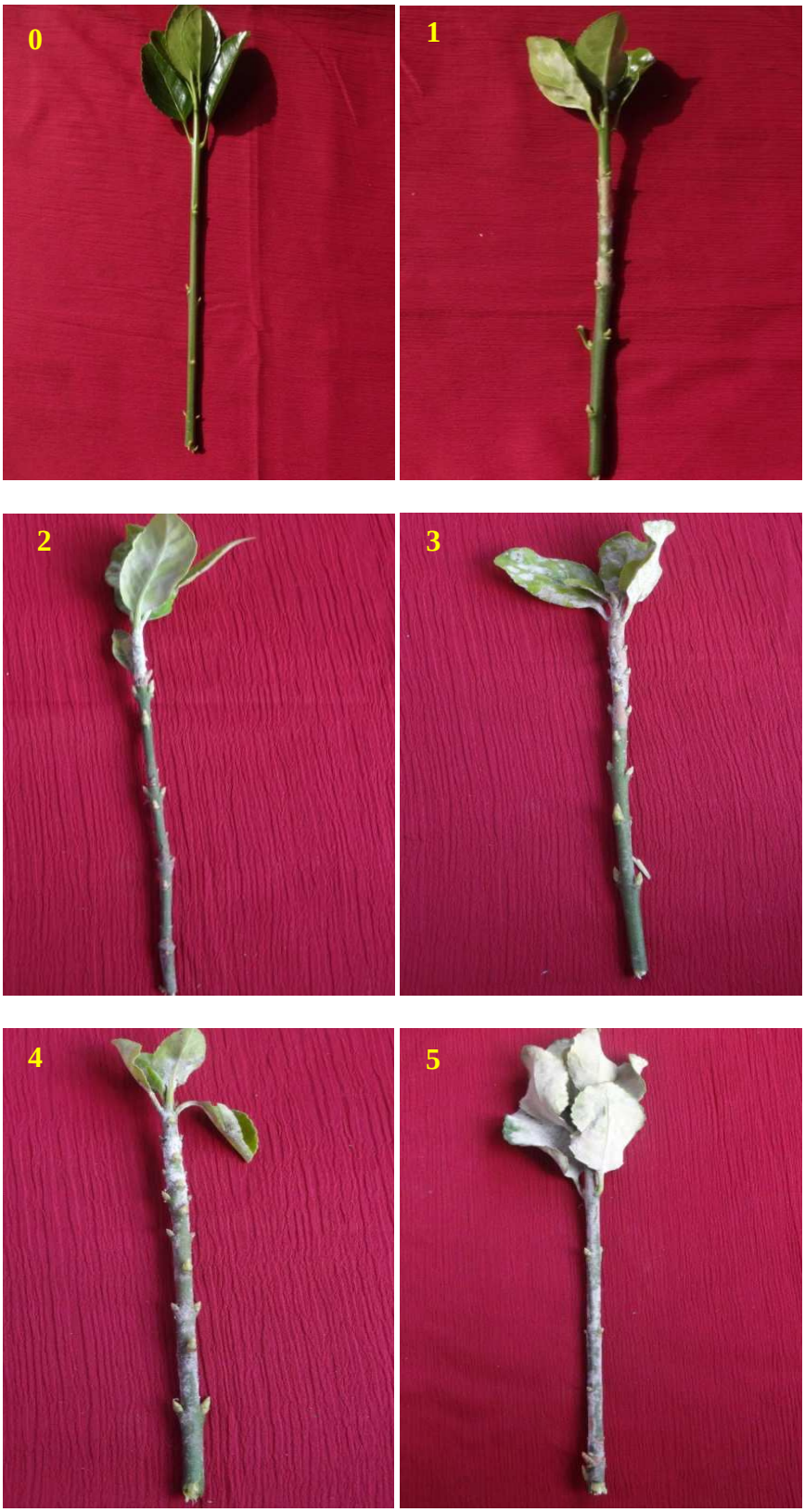


Plate-2: Assessment scale for disease intensity of powdery mildew of *Euonymus japonicus* caused by *Oidium euonymi-japonici*

Category	Numerical value	Leaf area/extension growth infected (%)
I	0	Disease free
II	1	Upto 25
III	2	>25 -50
IV	3	>50-75
V	4	>75
VI	5	Complete leaf fall

Per cent disease intensity (PDI) was calculated by using formula:

$$PDI = \frac{\Sigma (n \times v)}{N \times G} \times 100$$

Where,

Σ = Summation

n = Number of leaves/twigs in each category

v = Numerical value of each category

N = Total Number of leaves/twigs examined

G = Maximum numerical value

3.2 Pathogenicity test

Healthy *Euonymus japonicus* (cultivar Patens) twigs bearing leaves were identified and were cut with sterilized scissor, surface sterilized and immediately put in sterilize polybags containing sterilized soil. Ten such twigs were inoculated by rubbing the infected twigs of *E. japonicus* cultivar Patens and incubated in humid chamber. Uninoculated twigs were also maintained as control. Observations were made at regular intervals for symptom development. The symptoms developed on the inoculated twigs after 7 days of inoculation and these

infected twigs were then compared with the twigs used for the inoculation with respect to morphological characteristics of the pathogen.

3.3 Morphological characteristics

The morphological characteristics of the causal organism were studied on the host under laboratory conditions. Semi-permanent slides were prepared, and were examined under microscope (40X, 100X) with respect to following characters of the causal organism:

Mycelium	:	Colour, width, septation and branching
Conidiophores	:	Colour, shape, size and branching
Conidia	:	Colour, shape, size and septation

3.4 Symptomatology

Symptom development of powdery mildew was studied on *E. japonicus* plants tagged at Shalimar campus under natural epiphytotic conditions. The plants were observed constantly for symptom development. The diseased leaves were tagged and further observations with respect to size, shape, colour, coalescing and sporulation were recorded at weekly intervals till the spots attained the maximum possible size.

3.5 Perpetuation studies

The perpetuation of the pathogen on the intact infected twigs and leaves of *E. japonicus* was studied during 2013 and 2014 in an experimental field of the Division of Plant Pathology at SKUAST-K, Shalimar campus.

3.5.1 Survival on overwintered diseased leaves/twigs

The study was carried out on the intact twigs/leaves and on the cut diseased twigs kept in wire bags by placing them at a depth of 10 cm in the soil. The diseased twigs were collected on 1st November in the year 2012 and 2013.

The diseased twigs were kept in wire mesh bags, each bag containing equal number of twigs and kept 10 cm in the soil. The diseased twigs were removed from the wire mesh bags at weekly intervals beginning from 1st week of February onwards and examined in the laboratory for the production and viability of conidia. The equal number of intact infected twigs (10 twigs/plant) were also tagged on 1st November 2012 and 2013 and were observed from the 1st week of November 2012 to 1st week of November 2013 and from 1st week of November 2013 to 1st week of November 2014 respectively for the production and viability of conidia.

3.5.2 Conidial production

For the estimation of conidial production on diseased leaves a method given by Filajdec and Sutton (1995) was adapted. Twenty leaf discs of 1cm^2 dia bearing the mildewed symptoms were randomly selected, crushed in 80 ml distilled water in a pestle and Mortar and stained through a double layer of cheese cloth. 25 ml of this suspension was centrifuged at 6000 rpm for 15 minutes. After centrifugation, 18 ml of the supernatant was drawn off from the pippete. The pellet was re-suspended in 2 ml sterilized water and the number of conidia estimated as mean of 10 haemocytometer readings for replication. The number of conidia was computed on the basis of number per unit area

3.5.3 Conidial viability

Viability of conidia was studied by spore germination test of the overwintered leaves. Two drops of 50 µl from each processed sample were placed on a glass slide and incubated in a moist chamber at 25±1^{o}C. After 24 hour incubation, a drop of cotton blue in lactophenol was added to each drop and semi-permanent slides prepared by placing a 20 × 20 mm cover slip over each drop. The number of conidia in each drop and the number that germinated were recorded.

3.6 Host range studies

The objective of this study was to ascertain the host specificity of *Oidium euonymi-japonici* other than *E. japonicus*. Different Euonymus species (*E. japonicus, E. aureopictus, E. albomarginatus, E. microphyllus, E. fortunii* and *E. aureaomarginatus*), weeds (*Trifolium repens, Plantago major, P. minor, Convolvulus arvensis, Raninculus acris, Cynodon dactylon*) besides surrounding hedges/plants (*Rosa indica* and *Ligustrum ovulifolium*) were raised in sterilized pots containing sterilized soil under protected conditions. These were inoculated artificially on 1st of April by dusting the conidia collected from *E. japonicus* leaves naturally infected by powdery mildew by using hair brush. Inoculated plants were covered with polythene bags for 24 hours. Observations were recorded 10 days after inoculation on the development of symptoms on different hosts. Isolates were collected from different hosts other than *E. japonicus* as mentioned above infected by powdery mildew in the field. The conidia from these hosts were inoculated onto the healthy *E. japonicus* leaves by dusting conidia with camel hair brush. Similarly conidia from naturally infected *E. japonicus* leaves were inoculated on to the weeds and other euonymus species grown in pots. Observations for infection were then recorded to know the cross inoculable nature of the pathogen.

3.7 Epidemiological Studies

The epidemiological work has been studied at SKUAST-K Shalimar campus during 2013 and 2014. Ten plants were randomly selected and were labeled for observation on the powdery mildew disease development on foliage. Observations were recorded using 0-5 scale (Mirza, 1999) at weekly interval of standard meteorological week.

3.7.1 Inoculation and disease rating

The conidia *of Oidium euonymi-japonici* were dusted on the *E. japonicus* on the newly emerged leaves. The Percent disease intensity (PDI) was calculated

from 2nd week of April till peak period of the disease (4th week of June). Rate of disease progress was calculated using the given below (Vanderplank, 1963):

$$R = \frac{2.3}{T_2 - T_1} \log_{10} \frac{X_2}{X_1}$$

Where, r = rate of disease progress or infection rate.

$T_2 - T_1$ = Time interval

X_1 = Disease at time T_1

X_2 = Disease at time T_2

The PDI was correlated with meteorological data collected from the observatory of Agro-Meteorology, Section of the University SKUAST-Kashmir, Shalimar. The effect of weather parameters on powdery mildew of *E. japonicus* was correlated using SPSS (Statistical Package of Social Sciences). The regression and coefficient of multiple determination (R^2) values were also analysed to find out the effect of single as well as combination of different meteorological factors on disease development.

3.7.2 Correlation analysis

To determine the effect of weather variables on the progress of disease, the data on disease intensity during the course of epidemic was analyzed by calculating simple and multiple correlations (Gomez and Gomez, 1984).

3.7.3 Simple correlation

All simple correlations were calculated between study variables Y and abiotic factors X_1, X_2, X_3…..X_5. PDI, rainfall, relative humidity maximum and minimum, temperature maximum and minimum is represented by Y, X_1, X_2, X_3, X_4 and X_5, respectively. The test of significance for simple correlation was carried out using Student's t- test (Snedecor and Cochran, 1986).

3.7.4 Multiple correlation and regression analysis

To study the cumulative effect of different variables (independent variables) in disease development (PDI), multiple correlation coefficient were carried out and linear multiple regression model was developed for prediction of disease development. The coefficient of multiple determinations was used for the adequacy of regression equations.

3.7.6 Regression model

Linear multiple regression analysis were used to find out the effect of various environmental factors on disease progress by using prediction equation as:

$$Y = b_0 + b_1X_1 + b_2X_2 + \ldots\ldots\ldots\ldots + b_5X_5$$

3.8 Screening

Totally 6 cultivars were screened under artificial epiphytotic conditions at Division of Plant Pathology, SKUAST-Kashmir, Shalimar, during 2013. 10 plants of each cultivar were raised polybags and artificial inoculation was done by rubbing the infected leaves and twigs on healthy young leaves. All the leaves of 10 plants of each cultivar were counted for the assessment of the disease. Same plants were assessed each year and the average PDI of two years was taken for the categorization of the species. The disease was recorded during the peak period of the disease (4^{th} week of June). The species were categorized in 6 different groups in accordance with Datar (1922) as under:

Grade	Reaction	PDI
0	Immune (I)	Disease free
1	Highly resistant (HR)	Upto 10
2	Resistant (R)	>10-20
3	Moderately resistant (MR)	>20-40
4	Moderately susceptible (MS)	>40-60
5	Susceptible (S)	>60-100

3.9 Management

3.9.1 *In vivo* evaluation of fungicides

The field trial was conducted for two consecutive years during 2013 and 2014 at SKUAST-Kashmir Shalimar, during 2013. The experiment was laid out in Randomized Block Design with three replications and ten treatments. Four sprays of all treatments were undertaken immediately after the appearance of the disease at an interval of 15 days. The observations on disease intensity of powdery mildew were recorded 15 days after the last spray, on five randomly selected plants in each treatment. Three branches were randomly tagged per plant and the PDI of powdery mildew on all the leaves and twigs of these tagged branches were graded by adopting 0 to 5 scale as given by Mirza (1999). The fungitoxicants evaluated are as under:

S. No.	Commercial name	Treatment	Conc. (%)
1	Karathane	Dinocap 48 EC	0.05
2	Sulfex	Wettable sulphur 80 WP	0.20
3	Potassium bicarbonate	KHCO3	1.00
4	Tilt	Propiconazole 25 EC	0.03
5	Bayleton	Triadimefon 25 WP	0.05
6	Graple	Myclobutanil 10 WP	0.03
7	Score	Difenconazole 25 EC	0.03
8	Contaf	Hexaconazole 5 EC	0.03
9	Governar	Flusilazole 40 EC	0.02
10	Check (Water spray)	-	-

3.9.2 *In vivo* evaluation of biocontrol agents

The biocontrol agents namely *Trichoderma viride*, *Pseudomonas*

fluorescens and *Ampelomyces quisqualis* were procured from the Division of Plant Pathology, SKUAST-Kashmir, Shalimar. Culture filtrates of the biocontrol agents were obtained by growing biocontrol agents *T. viridi* and *Pseudomonas fluorescens* in PDA broth for 15 days at 26-28^{o}C. The PDA broth with biocontrol agents were then centrifuged at 5, 000 rpm for 15 minutes, the supernatant was filtered by filter glass (El-Boghdady, 1993). The filtrates were diluted at 25 and 50 v/v by sterilized water. Concentrations of 25 and 50 per cent were also prepared from the commercial biogent *A. quisqualis*. Culture filtrates of *T. viride* and *P. fluorescens* from concentrations 25 and 50 per cent and 25 and 50 per cent concentrations from commercial biogent *A. quisqualis* were used on the potted plants grown at research fields, Division of Plant Pathology during the two successive years 2013 and 2014 for their efficacy against the powdery mildew. The plants were sprayed four times from 10th of April at 15 days interval.

Observations regarding PDI were recorded 20 days after the last spray as per the formula mentioned in 3.1

3.9.3 Effect of Pruning on disease

The effect of pruning on the PDI of powdery mildew was studied on highly susceptible cultivar 'Patens' of *E. japonicus*. The different pruning levels taken were:

Treatment	Pruning levels
P_1	2 cm below diseased site
P_2	4 cm below diseased site
P_3	6 cm below diseased site
P_0	No pruning

3.10 Integrated disease management

The most effective fungitoxicant, biocontrol agent and a pruning level at

the respective recommendations were applied on the cultivar 'Patens' of *E. japonicus* at SKUAST-Kashmir, Shalimar, Srinagar. The experiment was laid in Randomised Block Design (RBD). The chemical and biocontrol agent was applied three times alternately at an interval of fifteen days before the appearance of symptoms on the new growth. Pruning was done on 28th February. Data on PDI was calculated 15 days after the last spray. The treatments given are as under:

Treatments	Treatment combinations
T_1	Fungitoxicant + Biocontol agent
T_2	Pruning + Fungitoxicant
T_3	Pruning + Biocontol agent
T_4	Pruning + Fungitoxicant+ Biocontol agent
T_5	Check (water spray)

3.11 Statistical analysis

The data obtained in different experiments were statistically analysed with the help of computer software SPSS, Minitab, OP Stat and Microsoft Excel. The data was subjected to appropriate transformations wherever needed, as suggested by Gomez and Gomez (1984) before analysis.

Chapter-4

EXPERIMENTAL FINDINGS

Experimental findings in the present investigations are elucidated under the following sub-headings:

4.1 Survey

Survey was conducted in three zones *viz*., central, north and south Zones of Kashmir valley to assess the status of powdery mildew of *Euonymus japonicus* during 2012 and 2013. The data regarding disease incidence and Per cent Disease Intensity (PDI) recorded are presented in Table 1 and 2.

4.1.1 Disease incidence on leaves

The results obtained indicate that powdery mildew of *E. japonicus* was prevalent in all the three zones surveyed. The disease incidence of 77.60 per cent was recorded on leaves in 2013 compared to 75.14 per cent in 2012 (Table-1). The disease incidence on leaves during the year 2012 was higher in south zone (76.99%) followed by central zone (75.84%) and least in north zone (72.41%). Disease incidence followed the similar trend in 2013.

Perusal of data revealed that average disease incidence on leaves varied from 56.67 to 86.70 per cent with Confidence Interval (95%) of 71.25-81.33. Among the zones surveyed the highest disease incidence was recorded in south zone (78.06%) followed by central zone (77.00%) and least in north zone (73.83%). Amongst the locations surveyed, Nishat in central zone recorded the highest average disease incidence (86.70%) whereas, and lowest average disease incidence (56.67%) was recorded at Iqbal Park of the same zone. In south zone the highest average disease incidence of 79.47 per cent was recorded at Verinag followed by Kokernag (78.35%), whereas, lowest was recorded at Achabal (76.64%). Similarly in north zone the highest average disease incidence of 79.25

percent was recorded at Tangmarg followed by Baramullah (77.55%) whereas, least was recorded at Manasbal (69.05%).

4.1.2 Per cent disease intensity on leaves

The overall per cent disease intensity (PDI) of 45.05 was recorded in 2013 as compared to 42.60 in 2012 (Table-1). In 2012, the PDI was higher in south zone (46.68%), followed by central zone (42.60%). Least PDI (38.50%) was recorded in north zone. PDI followed the similar trend in 2013.

Perusal of data revealed that the average PDI on leaves ranged from 53.33 to 20.89 with Confidence interval (95%) of 38.26-49.45. Amongst the zones surveyed highest average PDI (48.01%) was recorded at south zone followed by central zone (43.43%) and least was recorded at north zone (40.02%). Amongst the locations surveyed highest average PDI of 53.33 was recorded at Nishat of south zone, whereas lowest was recorded at Iqbal Park (20.89%). In South zone highest average PDI of 49.26 was recorded at Verinag followed by Kokernag (48.92%), whereas lowest was recorded at Achabal (46.00%). Similarly in north zone the highest average PDI of 43.11 was recorded at Tangmarg followed by Baramullah (41.68%) whereas, lowest was recorded at Manasbal (36.80%). The disease incidence on twigs was less as compared to disease incidence on leaves. The disease incidence on twigs was less in 2012 as compared in 2013. The overall disease incidence of 22.74 per cent was recorded in 2013 in comparison to 21.32 per cent in 2012 (Table 2). The disease incidence in 2012 was higher in south zone (23.01%) followed by central zone (22.57%). Least disease incidence of 18.38% was recorded north zone. A similar trend in disease incidence was observed in 2013.

Table 1: Status of Powdery Mildew of *Euonymus japonicus* on leaves in Kashmir valley during 2012-2013

Zone	Location	*Disease Incidence (%)			*Disease Intensity (%)		
		2012	2013	Pooled	2012	2013	Pooled
Central Zone	Harwan	80.83	83.13	81.98	48.39	50.78	49.58
	Shalimar	81.80	83.50	82.65	49.10	51.73	50.41
	Nishat	85.33	88.07	86.70	52.04	53.63	53.33
	Iqbal Park	55.40	57.93	56.67	20.85	20.93	20.89
Sub-Mean		**75.84**	**78.16**	**77.00**	**42.60**	**44.27**	**43.43**
CI (95%)		**53.93-97.74**	**56.40-99.91**	**55.17-98.82**	**19.39-65.79**	**19.43-69.09**	**19.41-67.45**
South Zone	Achabal	76. 40	78.90	76.64	44.67	47.33	46.00
	Kokernag	77.00	79.70	78.35	46.33	51.51	48.92
	Verinag	78.67	80.27	79.47	48.96	49.56	49.26
	Aharbal	76.93	78.62	77.77	46.79	48.97	47.88
Sub-Mean		**76.99**	**79.12**	**78.06**	**46.68**	**47.04**	**48.01**
CI (95%)		**75.68-78.81**	**78.17-80.57**	**76.18-79.93**	**43.87-49.50**	**46.59-52.08**	**45.68-50.34**
North Zone	**Sopore**	68.60	70.39	69.49	37.65	39.33	38.49
	Baramullah	75.33	79.77	77.55	39.44	43.93	41.68
	Tangmarg	78.47	80.03	79.25	41.35	44.88	43.11
	Manasbal	67.27	70.83	69.05	35.58	38.03	36.80
Sub-Mean		**72.41**	**75.25**	**73.83**	**38.50**	**41.54**	**40.02**
CI (95%)		**63.88-80.94**	**66.71-83.79**	**65.37-82.29**	**34.57-42.43**	**36.18-46.90**	**35.42-44.61**
Overall Mean		**75.14**	**77.60**	**76.37**	**42.60**	**45.05**	**43.82**

Table 2: Status of Powdery Mildew of *Euonymus japonicus* on twigs in Kashmir valley during 2012-2013

Zone	Location	*Disease Incidence (%)			*Disease Intensity (%)		
		2012	2013	Pooled	2012	2013	Pooled
Central Zone	Harwan	23.25	25.18	24.21)	13.23	14.13	13.68
	Shalimar	28.66	30.66	29.66	15.85	17.35	16.6
	Nishat	30.35	32.20	31.27	18.40	19.20	18.80
	Iqbal Park	8.03	8.35	8.19	3.35	3.90	3.62
Sub-Mean		**22.57**	**24.10**	**23.34**	**12.71**	**13.65**	**13.18**
CI (95%)		**6.41-38.73**	**6.71-41.47**	**6.56-40.10**	**2.22-23.18**	**2.78-24.50**	**2.50-23.84**
South Zone	Achabal	19.90	20.72	20.31	10.67	11.50	11.08
	Kokernag	21.65	22.18	21.91	11.25	12.67	11.96
	Verinag	24.18	24.95	24.56	13.55	14.00	13.77
	Aharbal	23.30	23.85	23.57	12.33	13.75	13.04
Sub-Mean		**22.25**	**22.92**	**22.59**	**11.95**	**12.98**	**12.46**
CI (95%)		**19.25-25.26**	**19.96-25.88**	**19.61-25.57**	**9.93-13.96**	**11.16-14.79**	**10.58-14.35**
North Zone	Sopore	19.00	20.35	26.29	9.65	10.55	10.10
	Baramullah	17.85	19.28	18.57	10.13	11.25	10.69
	Tangmarg	20.55	22.33	21.44	10.93	12.35	11.64
	Manasbal	16.13	17.80	16.97	9.13	10.30	9.72
Sub-Mean		**18.38**	**19.94**	**19.16**	**9.9**	**11.11**	**10.54**
CI (95%)		**15.41-21.35**	**16.90-22.97**	**16.17-22.15**	**8.74-11.17**	**9.65-12.57**	**9.20-11.86**
Overall Mean		**21.07**	**22.32**	**21.70**	**11.54**	**12.58**	**12.06**

*Average of 3 sites

4.1.3 Disease incidence on twigs

The data obtained revealed that the average disease incidence on twigs ranged from 8.19 to 31.27 per cent with Confidence Interval (95%) of 16.17-22.15. Amongst the locations surveyed, the highest average disease incidence of 31.27 per cent was recorded at Nishat of central zone and lowest at Iqbal Park (8.19%). In south zone the highest average disease incidence was recorded at Verinag (28.57%) and lowest at Achabal (20.31%). In north zone the highest average disease incidence was recorded at Tangmarg (21.44%) and lowest at Manasbal (16.97%). Manasbal area of North zone recorded the lowest disease incidence on twigs after Iqbal Park among all the locations surveyed.

4.1.4 Per cent disease intensity on twigs

PDI on twigs was less in 2012 as compared to 2013. The overall PDI of 12.90 was recorded in 2013 in comparison to 11.79 in 2012 (Table-2). In 2012 the PDI on twigs was higher in central zone (12.71%) followed by south zone (12.70%). Least PDI (9.90%) was recorded in north zone. PDI followed the similar trend in 2013.

Perusal of data revealed that the average PDI on twigs ranged from 18. to 3.62 per cent with Confidence Interval (95%) of 9.20 to 11.86. Among the zones surveyed the highest average PDI of 13.18 was recorded at central zone followed by south zone (13.04%) whereas, least PDI (10.54%) was recorded in north zone. Among the various locations surveyed the highest PDI (18.80%) was recorded at Nishat of central zone whereas, lowest PDI was recorded at Iqbal Park (3.62%). In south zone highest average PDI was recorded at Verinag (13.77%) followed by Kokernag (13.04%) whereas, least was recorded at Achabal (11.08%). Similarly in north zone highest PDI was recorded at Tangmarg (11.64%) followed by Baramullah (10.69%) whereas, least was recorded at Manasbal (9.72%).

4.2 Pathogenicity test

The conidia from the infected twigs of *E. japonicus* cv. “Patens” were

inoculated on the healthy leaves by rubbing the infected leaves on both the surfaces of healthy leaves. The characteristic symptoms appeared on the leaves after 7 days of inoculation showing small whitish spots that gradually increased in size and covered both the surfaces of leaves (Plate-3).

4.3 Morphology of *Oidium euonymi-japonici* Acrang.(Sacc.)

The morphological characters of the pathogen were studied in laboratory and are presented in Table-3.

4.3.1 Microscopic characters

The mycelium of *O. euonymi-japonici* was branched, septate, hyaline (Plate-4-A) measuring 3.4-7.5 µm (average 5.25 µm) in width. The hyphae showed elongated and hooked appresoria (Plate-4-B). The conidiophores were aseptate, ubranched and hyaline (Plate-4-C) measuring 75.7-91.5×5.3-9.1 (average 83.9-7.8) in size. The conidiophores were with unswollen basal cell with conidial chain of 1-5 cells (Plate-4-D). The conidia were hyaline and elliptical (Plate-4-E). The average dimensions of 100 elliptical conidia were 26.3-39.5 ×8.7-12.3 µm (average 33.9×10.8 µm).

4.4 Symptomatology

The symptomatological studies were carried out under field conditions on cultivar “Patens”. Ten plants were tagged and kept unsprayed throughout the season. The symptoms first appeared as chlorotic spots on the upper side of leaves in the second week of April and reached at its peak during the 1st week of July (Table-4). During this period the disease were also noticed on twigs. The symptomatological developments were recorded at an interval of seven days (Table-4). The first symptoms of the disease observed were whitish powdery tiny circular to irregular spots ranging from 2.0 to 3.5 mm with an average size of 2.9 mm





Plate-3: Pathogenicity: Symptom development of powdery mildew after 9 days of inoculation of *Oidium euonymi-japonici* on *Euonymus japonicus* cv. "Patens"

Table 3: Morphological characters of *Oidium euonymi-japonici* Acrang. (Sacc.)

	Shape	Size (µm)	Colour	Septation
Mycelium	Amphigeous, smooth, branched with hyphae having lobed appresoria	3.4-7.5 (average 5.25) in width	Hyaline	Septate
Conidiophore	Unbranched, erect, unswollen basal cell, with 1-5 conidial cells	75.7-91.5×5.3-9.1 (average 83.9-7.8)	Hyaline	Aseptate
Conidia	Elliptical usually in chains, vacuolated and germinating into germ tubes with lobed appresoria	26.3-39.5 ×8.7-12.3 (average 33.9×10.8)	Hyaline	Aseptate

The conidia were usually in chains, with vacuoles (Plate-4-F) and germinating into germ tubes with terminal lobed appresoria (Plate-4-G).

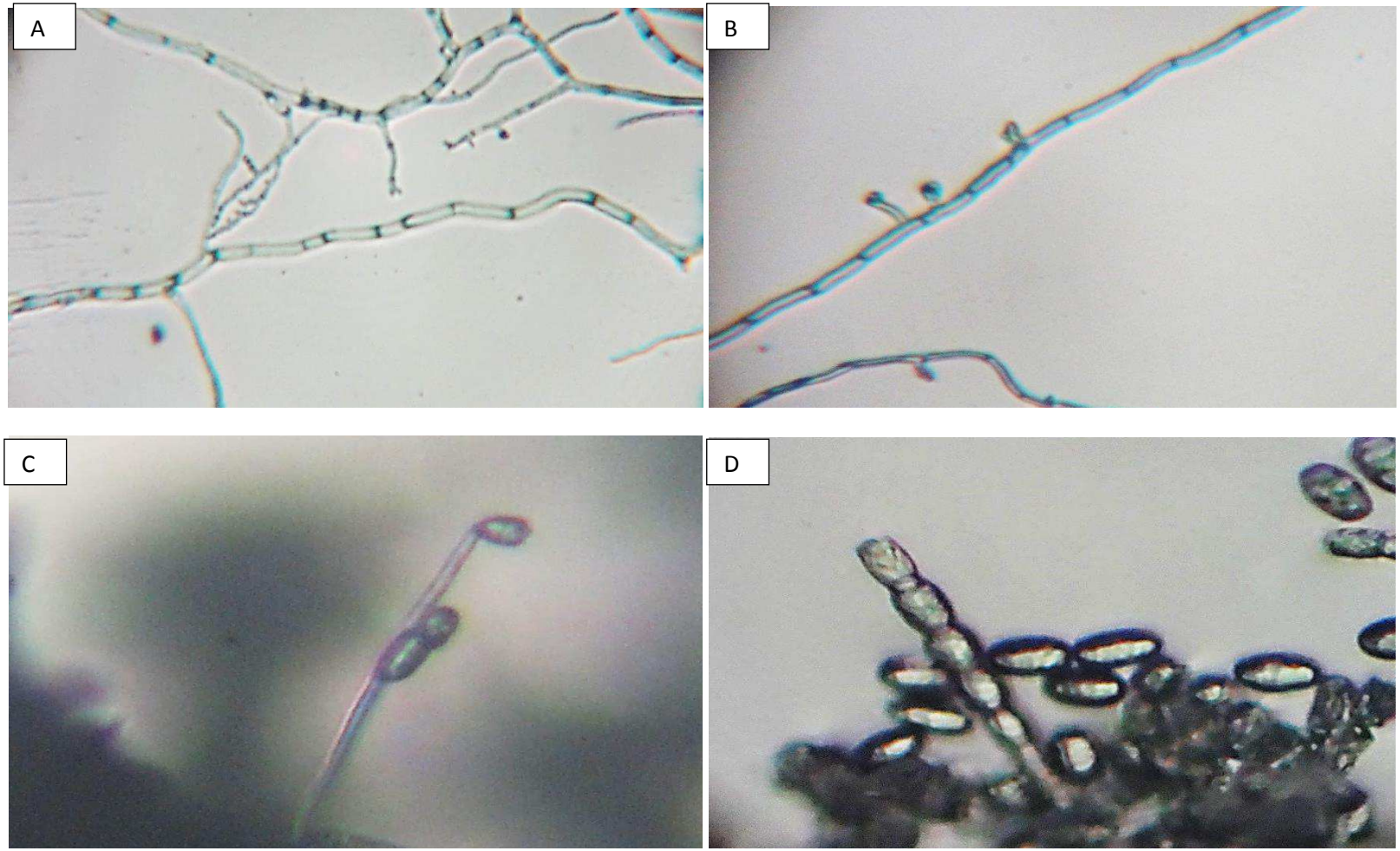
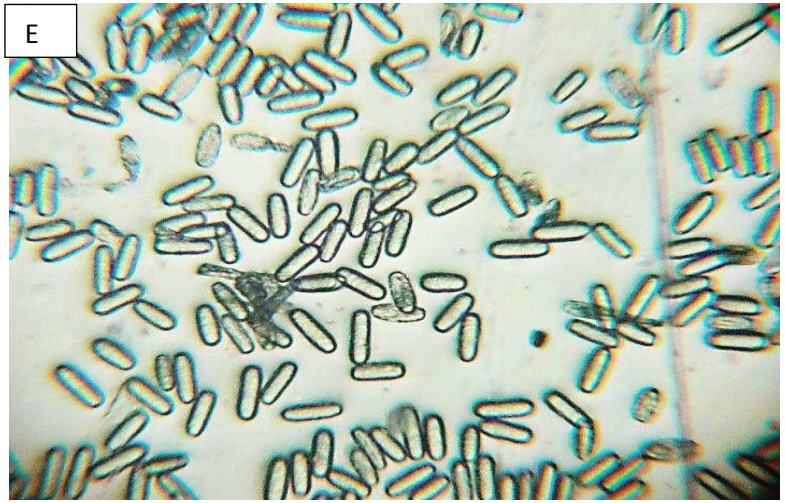


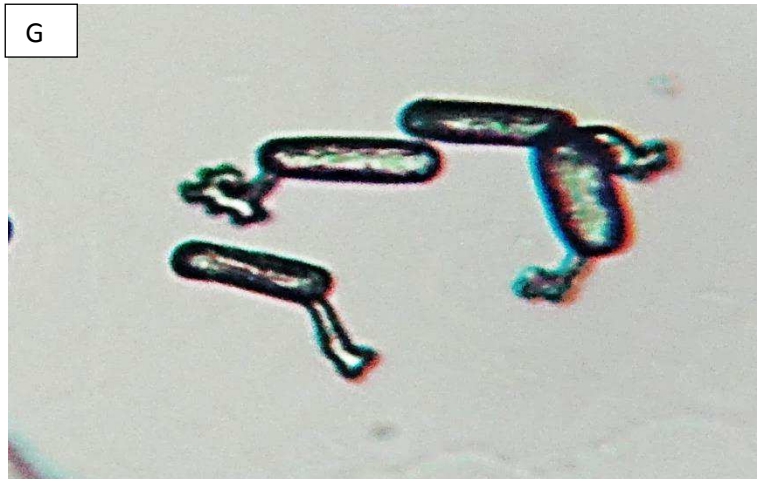
Plate-4: Morphology of *Oidium euonymi-japonici* Acrang. (Sacc.) on the *Euonymus japonicus* (100X)

A. **Mycelium**
B. **Hyphae with lobed aspersoria**
C. **Conidiphore with conidia**
D. **Conidiophore with conidia borne acropetally**

Plate-4 contd…….







E. Elliptical conidia
F. Vacuolated conidia
G. Germinating conidia with lobed appresoria

on the lower surface of leaves (Plate-5-A) in the second week of April. The whitish spots on the lower surface of leaves showed the yellowish chlorotic spots on the corresponding upper surface in the third week of April (Plate-5-B). White sporulating areas showing abundant production of conidia. In the fourth week of April, whitish circular to irregular spots start appearing on upper leaf surface (Plate-5-C). In the first week of May lesions started coalescing from both the sides of leaves to form bigger patches ranged from 4.9-7.2mm with an average of 6.8mm (Plate-5-D-E). In the 2nd week of May leaves showed mottled yellowing with patches of yellow and whitish growth (Plate-5-F-G). In the third week of May infection appeared on twigs as circular to irregular whitish growth (Plate5-H). Witches broom appearance of severely infected twigs appeared in the fourth week of May (Plate-8-I). Leaves become narrow and folded longitudinally in the first week of June (Plate-5-J). In the second and third weeks of June leaves become distorted, twisted and curled (plate-5-K). Leaves withered, shriveled, dried up and defoliate in the last week of June (Plate-5-L).

4.5 Host range of *Oidium euonymi-japonici*

The cross inoculation experiment was conducted to study the cross infectivity of powdery mildew pathogen from *E. japonicus* cv. Patens to other species of euonymus and other host plants under test and vice-versa (Table-5). In cross inoculation study, the pathogen *O. euonymi-japonici* from *E. japonicus* infected *E. aureopictus, E. albomarginatus, E. microphyllus, E. fortunii, E. aureaomarginatus* and vice-versa. Infected leaves showed the symptoms of yellowing on upper surface with whitish powdery mass consisted of conidiophores and conidia on their corresponding lower surface. *Trifolium repens, Plantago major, P. minor, Convolvulus arvensis, Raninculus acris, Cynodon dactylon, Rosa indica* and *Ligustrum ovulifolium* and different species of Euonymus did not show any symptoms of powdery mildew while cross inoculation studies (Plate-6).

Table 4: Development of symptoms of Powdery mildew on *E. japonicus* cv. Patens

Period of observation		Lesion						
		Size (mm)		Shape	Colour	Sporulation	Twig infection	Other characters
Month	Week	Range	Average					
April	II	1.1-2.3	1.8	Circular to irregular	Whitish	-	-	Whitish circular to irregular spots start appearing on lower surface of leaves
	III	2.0-3.5	2.9	-do-	-do-	+	-	Chlorotic spots appeared on the corresponding upper surface
	IV	3.5-5.5	4.3	-do-	-do-	+	-	Whitish, circular to irregular colonies of powdery growth appeared on the upper leaf surface
May	I	4.9-7.2	6.8	-do-	-do-	+	-	Lesions started coalescing from both the sides of leaves
	II	6.6-9.8	9.2	-do-	-do-	+	-	Leaves became mottled yellowing
	III	10.6-2.6	11.8	-do-	Whitish grey	+	+	Infection on twigs as small irregular patches of powdery growth
	IV	12.5-3.8	13.0	-do-	-do-	+	+	Witches broom appearance of severely infected twigs
June	I	13.2-4.9	14.1	-do-	-do-	+	+	Leaves became narrow and longitudinally folded
	II	14.7-5.7	15.0	-do-	-do-	+	+	Leaves become distorted , twisted and curled
	III	17.5-9.6	18.5	-do-	-do-	+	+	-do-
	IV	18.0-0.3	19.2	-do-	-do-	+	+	Leaves withered, shriveled and defoliate

+ denotes present and - denotes absent

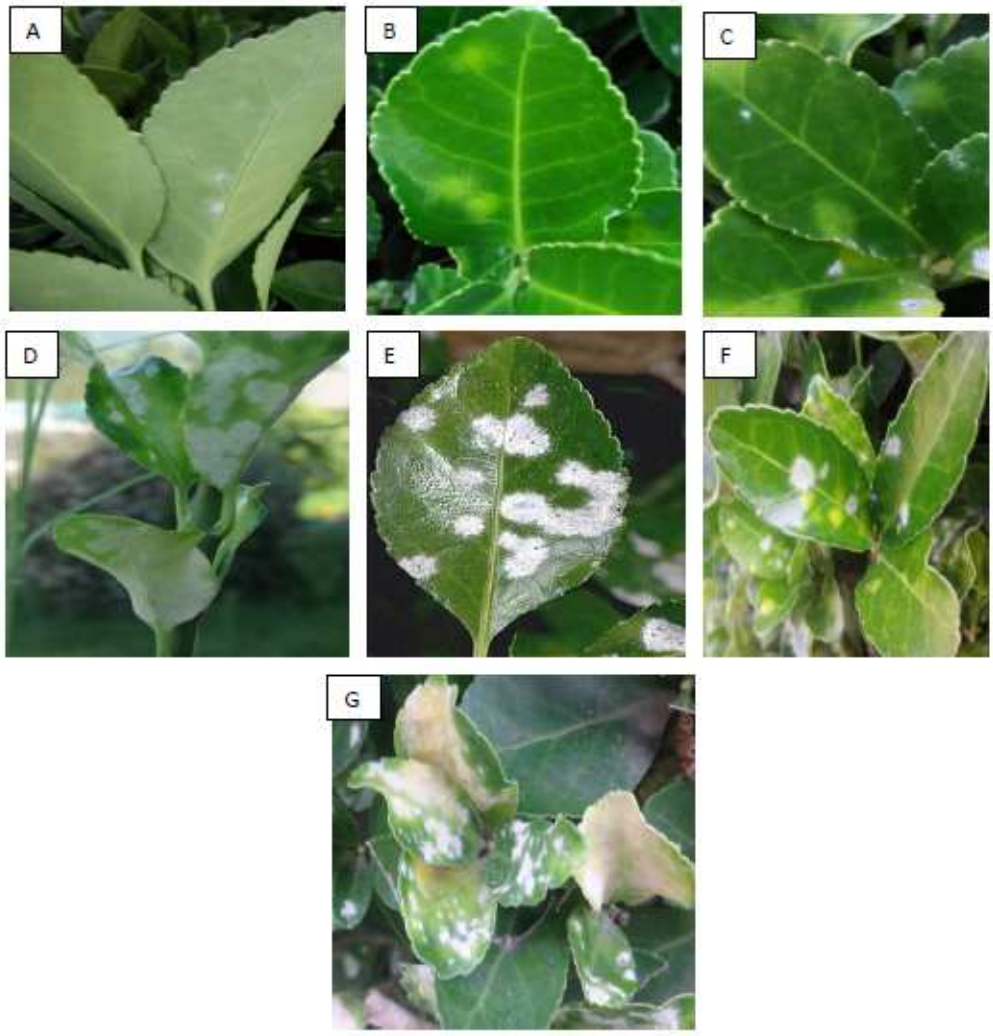


Plate-5: Symptomatological development of powdery mildew on *Euonymus japonicus*

A. Small dot like whitish powdery spots on lower surface
B. Chlorotic spots on corresponding upper leaf surface
C. Whitish lesions on upper leaf surface
D. Coalescing of spots on lower leaf surface
E. Coalescing of spots on upper leaf surface
F. Chlorosis and whitish circular spots on upper leaf surface

Plate -5 Contd…….











H. Powdery whitish growth on twig

I. Witches broom appearance

J. Longitudinal folding of leaves

K. Distorted, withered and shriveled leaves

L. Drying of severely infected leaves

4.6 Perpetuation of causal pathogen

The perpetuation studies of the causal pathogen (*Oidium euonymi-japonici*) were carried out on the intact infected twigs tagged in 1st week of November as well as on the infected twigs kept at 10cm deep in the soil during 2012 and 2013 and examined for conidila production and viability during 2013 and 2014.

4.6.1 Survival of *Oidium euonymi-japonici* on intact and overwintered diseased twigs

Observations regarding the conidial production and their viability were recorded at weekly intervals on the intact and overwintered diseased twigs and are presented in Table-5.

Perusal of data revealed that conidial production on the intact diseased twigs continued for a longer period from fourth week of February upto the 4th week of October in both the years of experimentation. The average conidial production and viability increased from 4rd week of February and reached maximum in the first week of April with production and viability of $4.4\times10^4 cm^{-2}$ of leaf area and 83.4% respectively. The average conidial production and viability decreased from the first week of April onwards and reached minimum in the first week of July with production and viability of $0.2\times10^4 cm^{-2}$ of leaf area and 15.6% respectively. Afterwards the favorable conditions again increased the average conidial production and viability which reached upto $4.3\times10^4 cm^{-2}$ of leaf area and 77.5% respectively in the first week of August and soon after it once again decreased. No conidial production and were observed on the intact infected twigs from the first week of November 2012 to 3rd week of February 2013 and from first week of November 2013 to 3rd week of February 2014. There was no conidial production on the diseased twigs kept at 10cm deep in the soil.

Table-5 : Host range of *Oidium euomymi-japonici* Acrang. (Sacc.)

S. No.	Host		Disease Reaction	**Leaf Disease Intensity (%)	Incubation period (days)
	Scientific name	Common name/ Local name			
1	*Euonymus aureopictus*	Variegated evergreen	+	13.67	9
2	*E. albomarginatus*	Variegated evergreen	+	17.55	9
3	*E. microphyllus*	Evergreen	+	9.70	9
4	*E. fortunii*	Evergreen	+	1.28	10
5	*E. aureaomarginatus*	Variegated evergreen	+	1.44	10
6	*Trifolium repens*	Tripater	-	No disease	-
7	*Plantago major*	Budgul	-	No disease	-
8	*P. minor*	Lakutgul	-	No disease	-
9	*Convolvulus arvensis*	Thraer	-	No disease	-
10	*Raninculus acris*	Meadow buttercup	-	No disease	-
11	*Cynodon dactylon*	Dramun	-	No disease	-
12	Rosa *indica*	Gulab	-	No disease	-
13	*Ligustrum ovulifolium*	Privet	-	No disease	-

\+ = Disease present

\- = Disease absent

Table 6 : Production and viability of *Oidium euonymi-japonici* on intact and overwintered diseased twigs of *E. japonicus* cv. "Patens" during 2013 and 2014

Period of observation		Intact twigs					
		*No. of conidia/cm^2 leaf area × 10^4			Viability (%)		
Month		**2013**	**2014**	**Mean**	**2013**	**2014**	**Mean**
February	3rd week	0.0	0.0	0.0	0.0	0.0	0.0
	4th week	1.3	1.1	1.2	21.8	19.0	20.4
March	1st week	1.6	1.3	1.5	25.0	27.9	26.5
	2nd week	2.2	1.6	1.9	56.5	44.5	50.5
	3rd week	3.8	3.2	3.5	60.8	59.6	60.2
	4th week	4.2	3.2	3.7	72.2	69.5	70.9
April	1st week	4.8	4.0	4.4	85.8	81.0	83.4
	2nd week	4.1	3.8	4.0	83.5	81.0	82.3
	3rd week	3.5	3.2	3.4	68.8	60.1	64.5
	4th week	3.2	2.6	2.9	65.6	55.2	60.4
May	1st week	2.8	2.3	2.6	53.1	50.0	51.6
	2nd week	2.0	1.8	1.9	42.0	40.0	41.0
	3rd week	1.3	1.2	1.3	31.27	0.0	15.6
	4th week	1.9	1.0	1.5	38.4	35.1	36.8
June	1st week	1.2	1.1	1.2	18.0	15.0	16.5
	2nd week	0.8	0.5	0.7	15.0	13.2	14.1
	3rd week	0.7	0.4	0.5	14.5	13.5	14.0
	4th week	0.5	0.3	0.4	14.2	13.1	13.7

Contd…

Table 6 contd…

Period of observation		Intact twigs					
		*No. of conidia/cm^2 leaf area × 10^4			Viability (%)		
Month		**2013**	**2014**	**Mean**	**2013**	**2014**	**Mean**
July	1st week	0.4	0.0	0.2	31.2	0.0	15.6
	2nd week	0.6	0.3	0.5	38.4	35.1	36.8
	3rd week	2.9	2.6	2.8	73.0	70.4	71.7
	4th week	3.3	3.1	3.2	75.3	71.1	73.2
August	1st week	4.2	4.4	4.3	79.2	75.8	77.5
	2nd week	3.6	3.1	3.4	45.0	38.3	41.7
	3rd week	2.1	1.9	2.0	38.1	34.5	36.3
	4th week	1.7	1.6	1.7	43.9	40.6	42.3
September	1st week	1.5	1.2	1.4	24.8	20.0	22.4
	2nd week	1.2	1.0	1.1	21.8	17.6	19.7
	3rd week	0.9	0.8	0.9	19.5	15.5	17.5
	4th week	0.7	0.6	0.7	25.0	22.9	24.0
October	1st week	0.6	0.4	0.5	21.8	19.0	20.4
	2nd week	0.4	0.3	0.4	15.0	13.0	14.0
	3rd week	0.3	0.3	0.3	0.0	0.0	0.0
	4th week	0.2	0.0	0.1	0.0	0.0	0.0

No conidial production was observed on the intact infected twigs from the first week of November 2013 to 3rd week of February 2014 and from first week of November 2013 to 3rd week of February 2015. No conidial production was observed on the twigs kept 10cm deep in the soil during both years of study.

4.7 Epidemiological studies

The effect of weather parameters *viz*., temperature (maximum and minimum), relative humidity (maximum and minimum) and rainfall (mm) on the development of powdery mildew (Per cent Disease Intensity) of *E. japonicus* was investigated under prevailing environmental conditions on susceptible cultivar "Patens", during 2013 and 2014. The data regarding per cent disease intensity were recorded at seven day intervals from 15th to 26th standard week (April-June) during 2013 (Table -7; figure-1) and from 16th to 26th standard week during 2014 (April-June) (Table-8; Fig. 2). The following observations were recorded.

During 2013, the powdery mildew symptoms were first observed on 15th standard week. The PDI increased slowly and reached as high as 56.93 during 26th standard week of 2013. The maximum infection rate of 0.1794 was recorded in the 17th standard week when the maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall during the previous week were 18.57 ^{o}C, 8.06 ^{o}C, 86.57%, 63.57 per cent and 3.83mm respectively followed by the infection rate of 0.1091 in the 18th standard week when the maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall during the previous week were 20.71 ^{o}C, 7.64 ^{o}C, 85.29 per cent, 48.14 per cent and 2.86 mm respectively. The minimum infection rate of 0.0005 was recorded in the 26th standard week when the maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall during the previous week were 31.29 ^{o}C, 18.11 ^{o}C, 78.00 per cent. 47.14 per cent and 3.89 mm respectively

In 2014 the maximum infection rate of 0.1398 was recorded in 19th standard week when the maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall during the previous week were 25.21^{o}C, 9.43 ^{o}C, 82.14 per cent, 51.00 per cent and 0.11 mm respectively followed by the infection rate of 0.1309 in the 18th standard week

when the maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall during the previous week were 25.21^{o}C, 7.56^{o}C, 79.86 per cent, 42.29 per cent and 0.00 mm respectively. The minimum infection rate of 0.0015 was recorded in the 26th standard week when the maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall during the previous week were 29.00 ^{o}C, 12.44 ^{o}C, 75.71 per cent. 46.00 per cent and 1.29 mm, respectively.

4.7.1 Correlation analysis

The analysis was made to establish the relationship between weather factors *viz*., maximum and minimum temperatures, maximum and minimum relative humidity and rainfall with PDI in highly susceptible variety 'Patens' through correlation and multiple linear regression analysis.

The relationship between PDI and weather factors during 2013 (Table-9) indicated a positive and significant correlation of disease intensity with the maximum and minimum temperatures with values of correlation coefficient (r) 0.853 and 0.863 respectively. The maximum and minimum relative humidity showed the negative though non-significant correlation with the PDI of powdery mildew with values of r equal to -0.083 and -0.463 respectively. The rainfall also showed negative non significant correlation with disease intensity (r=-0.033). Similarly during 2014 (Table 8) revealed that maximum and minimum temperature had significant positive correlation with correlation coefficients of 0.769 and 807 respectively. Maximum and minimum relative humidity indicated negative non significant correlation with correlation coefficients of -0.444 and -0.354 respectively. The rainfall also exhibited the non significant negative correlation with the disease intensity (r=-0.418). Analysis of pooled data revealed that the maximum and minimum temperature had significant positive correlation with disease intensity with correlation coefficients of 0.914 and 0.911 respectively.

Table 7: Mean weather parameters at different dates of observation and disease intensity of powdery mildew on leaves of *Euonymus japonicus* cv."Patens" during 2013

Standard week	Date of observation	Disease intensity (%)	Infection rate (R)	Temperature (^{o}C)		Relative humidity (%)		Rainfall (mm)
				Max.	Min.	Max.	Min.	
15th	13-04-13	0.96	-	20.50	5.29	65.86	58.71	1.40
16th	20-04-13	1.80	0.0897	19.29	6.16	82.57	55.00	7.14
17th	27-04-13	6.33	0.1794	18.57	8.06	86.57	63.57	3.83
18th	04-05-13	11.65	0.1091	20.71	7.64	85.29	48.14	2.86
19th	11-05-13	25.47	0.0437	22.43	8.44	83.71	50.00	0.77
20th	18-05-13	34.93	0.0243	22.36	9.63	84.43	60.14	3.06
21st	25-05-13	43.87	0.0576	30.14	10.43	69.43	35.14	0.00
22nd	01-06-13	48.33	0.0397	22.64	9.99	80.57	52.86	4.60
23rd	08-06-13	53.6	0.0249	30.21	12.34	78.00	49.57	1.86
24th	15-06-13	55.67	0.0108	30.71	16.37	79.29	50.71	6.14
25th	22-06-13	56.13	0.0057	25.71	12.36	80.71	53.14	3.09
26th	29-06-13	56.93	0.0005	31.29	18.11	78.00	47.14	3.89

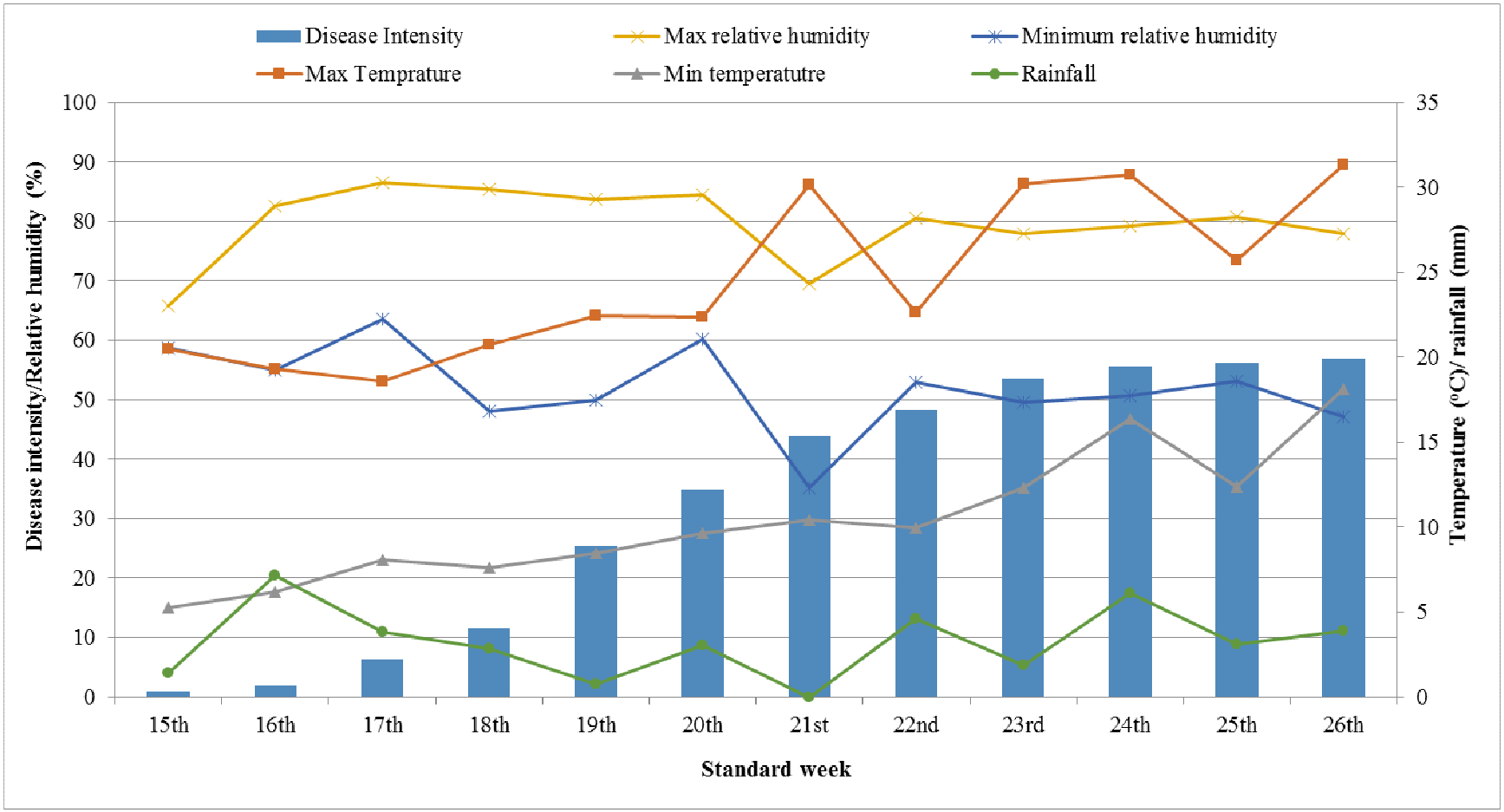


Fig. 1: Mean weather parameters at different dates of observation and per cent disease intensity of powdery mildew on leaves of *Euonymus japonicus* cv. "Patens" during 2013

Table 8 : Mean weather parameters at different dates of observation and disease intensity of powdery mildew on leaves of *Euonymus japonicus* cv. "Patens" during 2014

Standard week	Date of observation	Disease intensity (%)	Infection rate (R)	Temperature (^{o}C)		Relative humidity (%)		Rainfall (mm)
				Max.	Min.	Max.	Min.	
16th	20-04-14	1.04	-	14.07	6.23	82.71	76.14	7.60
17th	27-04-14	2.13	0.1023	21.07	6.70	70.43	52.29	0.74
18th	04-05-14	5.33	0.1309	25.21	7.56	79.86	42.29	0.00
19th	11-05-14	14.20	0.1398	25.21	9.43	82.14	51.00	0.11
20th	18-05-14	21.40	0.0585	18.57	9.00	86.14	64.29	6.20
21st	25-05-14	31.25	0.0540	21.21	8.86	85.00	63.29	3.53
22nd	01-06-14	39.66	0.0340	26.79	8.23	70.00	47.43	0.00
23rd	08-06-14	48.2	0.0278	27.83	9.54	72.43	51.00	2.06
24th	15-06-14	50.33	0.0062	30.57	9.56	62.86	39.86	0.00
25th	22-06-14	52.80	0.0068	29.01	13.34	76.71	42.57	0.10
26th	29-06-14	53.35	0.0015	29.00	12.44	75.71	46.00	1.29

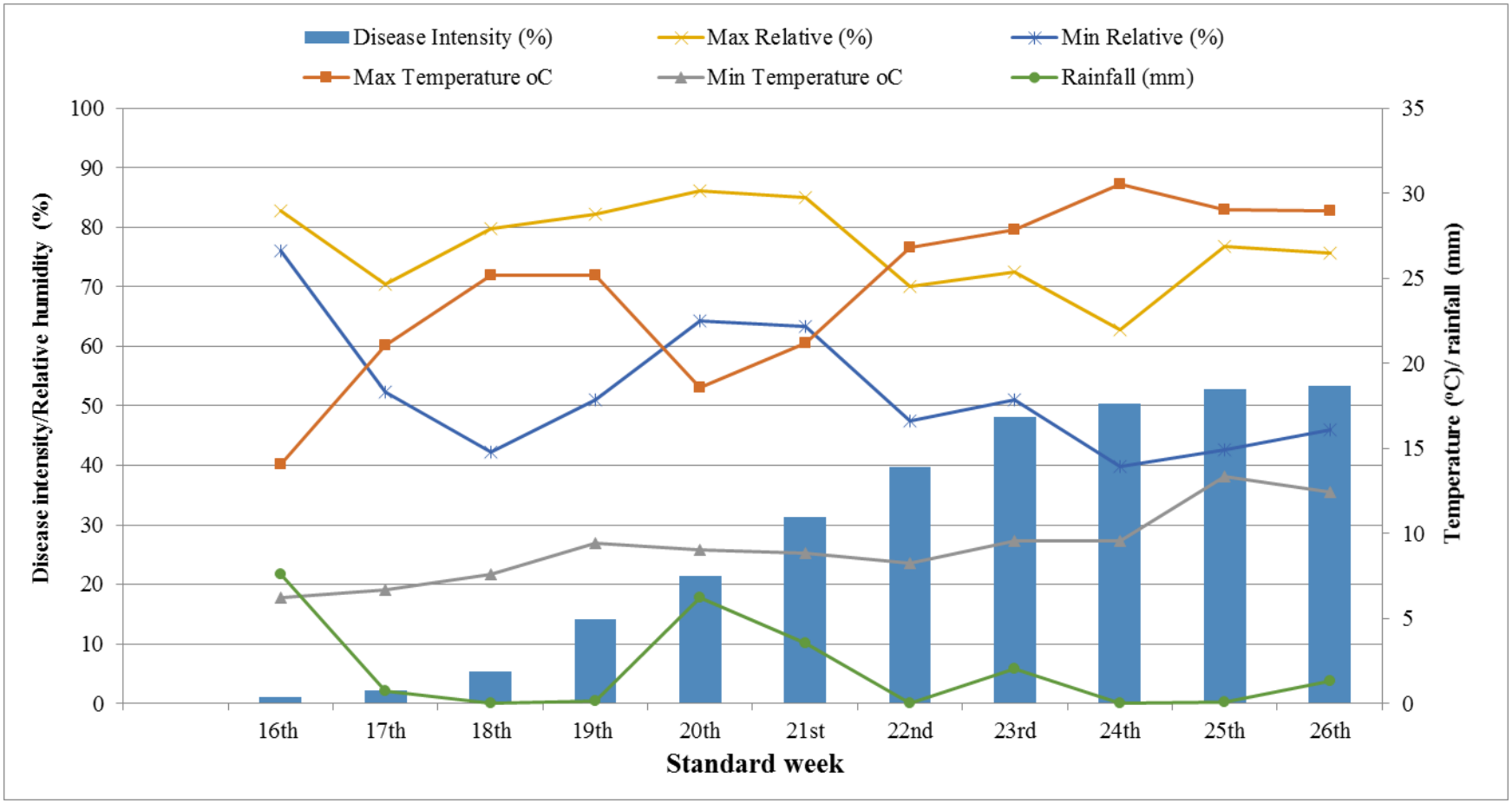


Fig. 2: Mean weather parameters at different dates of observation and per cent disease intensity of powdery mildew on leaves *Euonymus japonicus* cv. "Patens" during 2014

The analysis of the pooled data also revealed that minimum relative humidity had significant negative correlation with disease intensity with correlation coefficient of -0.664 and maximum relative humidity showed the negative non significant correlation. The rainfall showed the negative correlation with the disease intensity (r=-0.364).

4.7.2 Multiple linear regression analysis

The multiple linear regression of PDI of powdery mildew in relation to weather parameters, during 2013 (Table-10) indicated that, the regression coefficients for maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall were found to be 3.04, 2.05, 0.638, 0.228 and -1.156 respectively. Similarly in 2014, the regression coefficients for maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall were 5.41, 3.76, -0.772, 1.357 and 2.911 respectively. The pooled regression coefficients for both the years showed regression coefficients for weather parameters as maximum temperature (4.87), minimum temperature (1.80), maximum relative humidity (0.083), minimum relative humidity (1.203) and rainfall (-0.086).

During 2013, the multiple linear regression equation was fitted to the data and the equation arrived for the weather parameters is $\hat{Y}_1$- 122.10 + $3.04X_1$ + $2.05X_2$ + $0.638X_3$ + $0.228X_4$ - $1.156X_5$, whereas during 2014, the multiple linear regression equation was fitted as $\hat{Y}_2$ = -155.09+ 5.41 X_1+ $3.767X_2$ -0.772 X_3 +1.357 X_4 + 2.911 X_5. The pooled multiple linear regression equation for the both the years was fitted as $\hat{Y}$ = -174.60+ $4.87X_1$ +1.80 X_2 +$0.083X_3$ + 1.203 X_4 - 0.086 X_5. Where X_1 = maximum temperature (^oC) X_2 = Minimum temperature (^oC) X_3 = maximum humidity (%), X_4 =minimum humidity (%) and X_5 = Rainfall (mm).

This revealed that when there was increase in one unit of maximum and minimum temperature, maximum and minimum relative humidity and rainfall the

PDI was increased by 4.87, 1.80, 0.083, 1.203 units and decreased by 0.086 units respectively. The weather factors influence the disease intensity in *E. japonicus* cv. 'Patens' to the extent of 90 per cent.

4.8 *In vivo* evaluation of fungicides

A fungicide evaluation trial against powdery mildew was laid out on susceptible cultivar "Patens" at Shalimar campus SKUAST-K during 2013 and 2014. Eight systemic fungitoxicants and $KHCO_3$ were evaluated for their efficacy against powdery mildew under natural field conditions of disease development. The results are presented in Table-11

4.8.1 Disease intensity of powdery mildew on *Euonymus japonicus* cv. "Patens"

The analysis of data revealed that all the chemical treatments significantly reduced the disease both on leaves and twigs as compared to check during both the years (Table-11). The PDI among the chemical treatments ranged from 8.73 to 22.86 on leaves as compared to 60.26 per cent recorded in check and 1.44 to 6.65 on twigs as compared to 18.90 over the check. In 2014, the experiment was repeated and the results revealed that all the chemicals again proved effective in reducing the PDI on both leaves and twigs significantly over check. Among fungitoxicant treatments PDI ranged from 8.30 to 21.43 on leaves and 1.36 to 6.03 on twigs as compared to 58.80 and 18.05 recorded in check, respectively. Flusilazole 40 EC @ 0.02% proved significantly superior over all the treatments with the PDI of 8.30 and 1.36 on leaves and twigs, respectively followed by triadimefon 25 WP @ 0.05% (9.76% on leaves and 2.00% on twigs), but the later was statistically at par with difenconazole 25 EC @ 0.03% (10.06% on leaves and 2.21% on twigs) and propiconazole 25 EC @ 0.03% (10.73% on leaves and 2.37 on twigs) $KHCO_3$ was significantly effective in reducing the disease intensity on leaves and twigs (21.43 and 4.57%) as compared to check.

Table 9: Correlation coefficient between weather parameters and per cent disease intensity of powdery mildew on leaves of *Euonymus japonicus* cv."Patens"

S. No.	Weather parameter	Correlation Coefficient(ra)		
		2013	2014	Pooled
1	Maximum temperature (^oC)	0.853**	0.769**	0.914**
2	Minimum temperature (^oC)	0.863**	0.807**	0.911**
3	Max. relative humidity (%)	-0.083	-0.444	-0.434
4	Min. relative humidity (%)	.-0.463	-0.354	-0.664*
5	Rainfall (mm)	-0.033	-0.418	-0.364

. Correlation significant at 5% probability level

* Correlation significant at 1% probability level

Table 10: Multiple Regression model of per cent disease intensity

Year	Multiple correlation coefficient	Coefficient of multiple determination	Regression model
2013	0.901	0.812	$\hat{Y}_1 = -122.10 + 3.04X_1 + 2.05X_2 + 0.638X_3 + 0.228X_4 - 1.156X_5$
2014	0.970	0.940	$\hat{Y}_2 = -155.09 + 5.41\ X_1 + 3.767X_2 - 0.772\ X_3 + 1.357\ X_4 + 2.911\ X_5$
Pooled	0.950	0.902	$\hat{Y} = -174.60 + 4.87X_1 + 1.80\ X_2 + 0.083X_3 + 1.203\ X_4 - 0.086\ X_5$

$\hat{Y}_1$, $\hat{Y}_2$ = Disease intensity X_1 = Max. temperature (^oC) X_2= Min. temperature (^oC)

X_3 == Max Relative Humidity $X_{4=}$ Min Relative Humidity X_5 = Rainfall (mm)

* Significant at 1% probability level ** Significant at 5% probability level

Table 11: ***In vivo*** **effect of various fungicides on per cent disease intensity of powdery mildew of** ***Euonymus japonicus*** **cv."Patens" at Shalimar during the year 2013 and 2014**

Treatment	Conc. (%)	* Disease intensity (%)					
		Leaves			Twigs		
		2013	2014	Pooled	2013	2014	Pooled
Dinocap 48 EC	0.05	12.53 (22.72)	10.86 (19.22)	**11.70 (19.97)**	5.11 (13.04)	4.97 (12.85)	**5.04 (12.94)**
Wettable sulphur 80 WP	0.2	21.06 (27.30)	19.66 (26.29)	**20.36 (26.80)**	6.65 (14.91)	5.40 (13.40)	**6.03 (14.16)**
$KHCO_3$	1.00	22.86 (28.53)	20.00 (26.55)	**21.43 (27.54)**	5.91 (4.02)	3.23 (10.33)	**4.57 (12.17)**
Propiconazole 25 EC	0.03	11.60 (19.89)	9.86 (18.29)	**10.73 (19.09)**	2.70 (9.43)	2.04 (8.19)	**2.37 (8.81)**
Triadimefon 25 WP	0.05	10.33 (18.72)	9.20 (17.64)	**9.76 (18.18)**	2.03 (8.17)	1.97 (8.05)	**2.00 (8.11)**
Myclobutanil 10 WP	0.03	12.33 (20.53)	10.73 (19.11)	**11.53 (19.82)**	3.25 (10.36)	2.13 (8.37)	**2.69 (9.36)**
Difenconazole 25 EC	0.03	10.80 (19.17)	9.33 (17.77)	**10.06 (18.47)**	2.35 (8.79)	2.07 (8.25)	**2.21 (8.52)**
Hexaconazole 5 EC	0.03	12.93 (21.05)	12.26 (20.48)	**12.60 (20.77)**	3.55 (10.83)	2.97 (9.90)	**3.26 (10.37)**
Flusizole 40EC	0.02	8.73 (17.13)	7.86 (16.28)	**8.30 (16.70)**	1.44 (6.87)	1.28 (6.47)	**1.36 (6.67)**
Control	-	60.26 (50.90)	57.33 (49.19)	**58.80 (50.05)**	18.90 (25.71)	17.20 (24.45)	**18.05 (25.08)**
Mean		**18.34 (24.39)**	**16.71 (23.08)**		**5.19 (12.21)**	**4.33 (11.03)**	**(4.76)**

C.D $_{(p\leq 0.05)}$		**Leaves**	**Twigs**
Treatment	=	1.02	0.49
Year	=	0.46	0.21
Treatment×year	=	N.S.	0.68

*Mean of three replication; figures within parenthesis are arc sine transformed values and twigs respectively.

4.9 *In vivo* evaluation of biocontrol agents

In the present study cultural filtrates of three biocontrol agents viz., *Trichoderma viride, Pseudomonas fluorescens* and *Ampelomyces quisqualis* were evaluated at 25 and 50 per cent concentrations for their comparative efficacy in combating the disease under natural field conditions of disease development. The cultural filtrates of biocontrol agents were sprayed four times at an interval of 15 days on the susceptible cultivar "Patens" during the year 2013 and 2014.

4.9.1 Effect of culture filtrates on the per cent disease intensity of powdery mildew on leaves of *E. japonicus* cv."Patens"

The analysis of data (Table-12) revealed that all the biocontrol agents significantly reduced the disease intensity as compared to check.

Culture filtrates from the three selected biocontrol agents evaluated at two different concentrations (25 and 50%) against the powdery mildew of *E. japonicus* revealed that all the bioagents significantly reduced the PDI of powdery mildew of *E. japonicus* on leaves. During the year 2013, the PDI on leaves ranged from 35.60 to 41.17 against 59.46 in check. *A. quisqualis* was significantly effective over other biocontrol agents exhibiting PDI of 35.60 followed by *P.fluorescens* (40.01%) and *T. viride* (41.17%). In 2014 the experiment was repeated and the results revealed that biocontrol agents again proved effective in controlling the disease intensity significantly over the check.

Analysis of the pooled data (Table-13) revealed that all the biocontrol agents were significantly effective in controlling the PDI over the check. *A. quisqualis* was significantly effective over the other biocontrol agents with PDI of 34.71 followed by *P. fluorescence* (39.45%) and *T. viride* (40.53%).

Comparison of the concentrations *viz*., 25 and 50% of each bioagent revealed that *A. quisqualis* tested at two different concentrations showed PDI of 24.33 and 21.63 on leaves and twigs respectively followed by *P. fluorescens* with PDI of 31.06 and 29.11 and *T. viride* with PDI of 33.43 and 30.00 at two different

Table 12: ***In vivo*** **effect of various biocontrol agents on the per cent disease intensity of powdery mildew on leaves of** ***Euonymus japonicus*** **cv. "Patens" at Shalimar during the year 2013 and 2014**

Treatment	* Disease intensity (%)			Mean	* Disease intensity (%)			Mean	* Disease intensity (%)			Mean
	2013				2014				Pooled			
	Conc. (%)				Conc. (%)				Conc. (%)			
	0	25	50		0	25	50		0	25	50	
Trichoderma viride	59.46 (50.43)	34.00 (35.65)	30.06 (33.23)	**41.17 (39.77)**	56.86 (48.92)	32.86 (34.96)	29.93 (33.15)	**39.88 (39.01)**	58.16 (49.68)	33.43 (35.30)	30.00 (33.19)	**40.53 (39.39)**
Pseudomonas fluorescens	59.46 (50.43)	31.40 (34.06)	29.16 (32.67)	**40.01 (39.05)**	56.86 (48.92)	30.73 (33.64)	29.06 (32.61)	**38.88 (38.39)**	58.16 (49.68)	31.06 (33.85)	29.11 (32.64)	**39.45 (38.72)**
Ampelomyces quisqualis	59.46 (50.43)	25.00 (29.98)	22.33 (28.18)	**35.60 (36.20)**	56.86 (48.92)	23.66 (29.09)	20.93 (27.21)	**33.82 (35.07)**	58.16 (49.68)	24.33 (29.54)	21.63 (27.70)	**34.71 (35.64)**
Mean	**59.46 (50.43)**	**30.13 (33.23)**	**27.18 (31.36)**		**56.86 (48.92)**	**29.08 (32.56)**	**26.64 (30.99)**		**58.16 (49.68)**	**29.61 (32.90)**	**26.91 (31.18)**	

C.D$_{(p\leq 0.05)}$

Treatment (T)	0.48	0.69	0.55
Conc. (C)	0.48	0.69	0.55
Treatment×Conc.	0.84	1.19	0.96

*Mean of four replications; figures within parenthesis are arc sine transformed values

Table 13: ***In vivo*** **effect of various biocontrol agents on the intensity of powdery mildew on twigs of** ***Euonymus japonicus*** **cv.“Patens”at Shalimar during the year 2013 and 2014**

Treatment	* Disease intensity (%)				* Disease intensity (%)				* Disease intensity (%)			
	2013			Mean	2014			Mean	Pooled			Mean
	Conc. (%)				Conc. (%)				Conc. (%)			
	0	25	50		0	25	50		0	25	50	
Trichoderma viride	17.35 (24.59)	11.67 (19.95)	9.55 (17.98)	**12.86 (20.84)**	16.85 (24.21)	9.97 (18.38)	7.60 (15.98)	**11.47 (19.52)**	17.10 (24.40)	10.82 (19.18)	8.57 (17.00)	**12.17 (20.19)**
Pseudomonas fluorescens	17.35 (24.59)	10.67 (19.04)	9.20 (17.63)	**12.41 (20.42)**	16.85 (24.21)	8.9 (17.33)	7.20 (15.54)	**10.98 (19.03)**	17.10 (24.40)	9.79 (18.20)	8.2 (16.62)	**11.70 (19.74)**
Ampelomyces quisqualis	17.35 (24.59)	7.93 (16.33)	5.10 (13.03)	**10.13 (17.98)**	16.85 (24.21)	6.13 (14.31)	4.97 (12.86)	**9.32 (17.13)**	17.10 (24.40)	7.03 (15.35)	5.04 (12.94)	**9.72 (17.56)**
Mean	**17.35 (24.59)**	**10.09 18.44**	**7.95 (16.21)**		**16.85 (24.21)**	**8.33 (16.68)**	**6.59 (14.80)**		**17.10 (24.40)**	**9.21 (17.58)**	**7.27 (15.52)**	

C.D$_{(p\leq 0.05)}$

Treatment (T)	0.80	0.76	0.78
Conc. (C)	0.80	0.76	0.78
Treatment×Conc.	1.39	1.31	1.35

*Mean of four replications; figures within parenthesis are arc sine transformed values

concentrations respectively. The concentrations showed significant differences in reducing the PDI of all the biocontrol agents evaluated.

4.9.2 Effect of culture filtrates on the per cent disease intensity of powdery mildew on twigs

Analysis of data (Table-13) revealed that all the biocontrol agents significantly reduced the PDI on twigs as compared to check.

Culture filtrates from the three selected biocontrol agents evaluated at two different concentrations (25 and 50%) against the powdery mildew of *E. japonicus* revealed that all the bioagents significantly reduced the PDI of powdery mildew of *E. japonicus* on twigs. During the year 2013, the PDI on twigs ranged from 10.86 to 12.13 against 17.35 in check. During the year 2013, *A. quisqualis* was significantly effective in controlling the PDI on twigs over other biocontrol agents with PDI of 10.13 per cent followed by *P.fluorescens* (12.41%) and *T. viride* (12.86%) which were statistically at par with each other. In 2014 the experiment was repeated and the results revealed that biocontrol agents again proved effective in controlling the disease intensity significantly over the check.

Analysis of the pooled data (Table 13) revealed that all the biocontrol agents were significantly effective in controlling the PDI over the check. *A. quisqualis* was significantly effective over the other biocontrol agents with PDI of 9.72 followed by *P. f fluorescens* (11.70%) and *T. viride* (12.17%).

Comparison of the concentrations *viz*., 25 and 50% of each bioagent revealed that *A. quisqualis* tested at two different concentrations showed PDI of 7.03 and 5.04 on twigs respectively followed by *P.fluorescens* with PDI of 9.79 and 8.20 and *T. viride* with PDI of 10.82 and 8.57 at two different concentrations respectively. The concentrations showed significant differences in reducing the PDI of all the biocontrol agents evaluated.

4.10 Effect of pruning disease

The effect of different pruning levels on the PDI of powdery mildew of *E.*

japonicus were studied on the susceptible cultivar 'Patens' during 2013 and 2014 for their comparative efficacy in inhibiting the disease under natural field conditions of disease development. Pruning was performed in the month of February 2013 and 2014.

4.10.1 Effect of punning on the per cent disease intensity powdery mildew on leaves of *E. japonicus* cv. "Patens" at Shalimar.

The data presented in the Table 15 reveals that the pruning levels 2, 4 and 6 cm below the diseased region significantly reduced the PDI over the check. During, pruning level of 6cm below the diseased site was significantly superior over the other pruning levels with PDI of 19.04 followed by pruning level of 4cm with PDI of 28.85 and pruning level of 2 cm with PDI of 36.60. In 2014 the experiment was repeated and the results revealed that pruning levels (2, 4 and 6cm below diseased site) again proved effective in controlling the PDI significantly over the check.

Analysis of the pooled data (Table 15) revealed that all the pruning levels significantly reduced the PDI on leaves over the check. Pruning level of 6cm below the diseased site proved significantly effective over check and other pruning levels (2 and 4cm below diseased sit) in inhabiting the PDI by 18.79 followed by pruning 4cm below diseased site with PDI of 27.52 and pruning 2cm below diseased site with PDI of 36.20.

4.10.2 Effect of punning on the disease intensity of twigs powdery mildew of *E. japonicus* cv. "Patens"

It is evident from the data presented in Table 15 that during 2013 all the pruning levels significantly reduced the PDI of twigs over the check. The range of PDI in pruning levels 2, 4 and 6 cm below the diseased sites varied from 5.18 to 12.85. The pruning level of 6 cm below the diseased site was significantly superior over the other pruning levels with PDI of 5.18 followed by pruning level of 4 cm with PDI of 9.75 and pruning level of 2 cm with PDI of 12.85. In 2014 the experiment was repeated and the results revealed that pruning levels (2, 4 and 6 cm below diseased site) again proved effective in controlling the PDI significantly over the check.

Table-14 : Effect of various pruning levels on the intensity of powdery mildew of *Euonymus japonicus* cv."Patens" at Shalimar

Pruning level	* Disease intensity%			* Disease intensity%		
	Leaves			Twigs		
	2013	**2014**	**Pooled**	**2013**	**2014**	**Pooled**
P_1 (2 cm below diseased site)	36.60 (37.20)	35.80 (36.73)	36.20 (36.96)	12.85 (20.97)	11.13 (19.45)	11.99 (20.21)
P_2 (4 cm below diseased site)	28.85 (32.46)	26.20 (30.77)	27.52 (31.62)	9.75 (18.16)	8.46 (16.88)	9.11 (17.52)
P_3 (6 cm below diseased site)	19.04 (25.85)	18.54 (25.49)	18.79 (25.67)	5.18 (13.13)	4.97 (12.85)	5.08 (12.99)
P_0 (No pruning)	59.66 (50.55)	56.33 (48.62)	57.99 (49.59)	18.35 (25.32)	17.4 (24.61)	17.88 (24.97)
Total Mean	**31.04 (36.51)**	**33.36 (35.40)**		**11.53 (19.40)**	**10.49 (18.45)**	

$CD_{(p\leq 0.05)}$		Leaves	Twigs
Treatment	=	0.55	0.28
Year	=	0.39	0.20
Treatment×Year	=	0.78	0.39

*Mean of five replications; figures within parenthesis are arc sine transformed values

Analysis of the pooled data (Table 15) all the pruning levels significantly reduced the PDI on twigs over the check. Pruning level of 6cm below the diseased site proved significantly effective over check and other pruning levels (2 and 4cm below diseased sit) in inhabiting the PDI by 5.08 followed by pruning 4cm below diseased site with PDI of 9.11 and pruning 2cm below diseased site with PDI of 11.99. All the interaction means between the treatments showed significant difference with each other (Table 15)

4.11 Field reaction of different cultivars of *E. japonicus* cv. "Patens" against the powdery mildew

Six cultivars of *E. japonicus* were evaluated against powdery mildew during 2013 and 2014 under artificial inoculated conditions (Table-16). It is evident from the data presented in table 11 that different cultivars exhibited varying per cent disease intensities. The PDI was non significant in two years 2013 and 2015 with PDI of 17.97 and 16.91 per cent respectively. The PDI among the cultivars ranged from 1.13 per cent in Silver Queen to 59.13 in Patens during 2013 against 1.66 in Silver Queen to 56.66 in Patens in 2014. In the year 2013 the cultivar Patens was followed by Emerald Gaiety, Canadale Gold, Box leaf euonymus and Silver King with PDI of 18.35, 15.04, 11.13 and 3.04 respectively. Least PDI of 1.13 per cent was observed in Silver Queen. In 2014 the cultivars evaluated showed the similar pattern of disease development with PDI of 57.66, 16.83, 12.67, 10.70 and 1.93 in cultivars Emerald Gaiety, Canadale Gold, Box leaf euonymus, Silver King and Silver queen respectively. The analysis of the pooled data for the two years depicted that the cultivars show different reactions to the pathogen. Maximum PDI 58.40 was recorded in cultivar Patens which was significantly different from all the other cultivars tested. The cultivar Patens was followed by cultivars Emerald Gaiety, Canadale Gold, Box leaf euonymus, Silver King with PDI if 17.59 ,13.86, 10.92 and 2.49 respectively. Least PDI of 1.40 was recorded in cultivar Sliver Queen which was significantly at par with cultivar Silver King. The disease intensities of Emerald Gaiety and Canadale Gold were also statistically at par with each other and significantly different from all the other cultivars tested.

Table-15: Screening of *Euonymus japonicus* cultivars against powdery mildew

Cultivar	Disease Intensity (%)			Reaction*
	2013	2014	Pooled	
Patens	59.13 (50.30)	57.66 (48.85)	58.40 (49.57)	S
Canadale Gold	15.04 (22.77)	12.67 (21.65)	13.86 (22.21)	MR
Emerald Gaiety	18.35 (25.31)	16.83 (24.72)	17.59 (25.01)	MR
Box-leaf euonymus	11.13 (19.44)	10.7 (18.10)	10.92 (18.77)	MR
Silver King	3.04 (10.01)	1.93 (8.76)	2.49 (9.39)	R
Silver Queen	1.13 (6.08)	1.66 (7.38)	1.40 (6.73)	R
Total	**17.97 (22.32)**	**16.91 (21.58)**		

CD (P=0.05)

Cultivar	:	2.86
Year	:	N.S.
Cultivar × Treatment	:	N.S.

*Categorized as I-Immune (0% PDI), R- Resistant (upto10% PDI), MR- Moderately Resistant (>10-25%PDI)-moderately susceptible (>25-50% PDI), S-susceptible (>50-75% PDI) and HS-highly susceptible (>75% PDI)

Figures in parenthesis are arc sine transformed values for two years data

Of the cultivars screened Emerald Gaiety, Canadale Gold and Box-leaf euonymus were found to be moderately resistant, Silver King and Silver Queen as resistant while as the cultivar Patens was found to be susceptible in their reactions to the powdery mildew.

4.12 Integrated disease management in controlling powdery mildew of *E. japonicus* cv. "Patens" under *in vivo* conditions

The most effective fungitoxicant *viz*., flusilazole, biocontrol agent *viz*. *Ampelomyces quisqualis* and pruning level *viz*., 6 cm below the diseased site were applied to the susceptible cultivar 'Patens' during 2015 (Table-16). The pruning was done in the month of February (28^{th}). The biocontrol agent and fungitoxicant was sprayed alternatively three times at an interval of 14 days. All the treatments significantly reduced the PDI of powdery mildew. Pruning 6 cm below diseased site+ *Ampelomyces quisqualis* @ 50%+ flusilizole 48 EC @ 0.02% proved to be most effective in controlling the PDI on both leaves and twigs (2.33 and 0.16%) which was significantly superior over the check (56.40 and 17.15%) followed by pruning 6 cm below diseased site + flusilazole 48 EC @ 0.02% with PDI of 4.67 and 0.48 on leaves and twigs. Pruning 6 cm below diseased site+ *Ampelomyces quisqualis* @ 50% proved to be least effective in controlling the disease showing the PDI of 9.93 and 2.88 on leaves and twigs respectively. All the treatments differed significantly in reducing the disease intensity on both leaves and twigs.

Table-16: Integrated Disease management of powdery mildew of *Euonymus japonicus* cv. "Patens" during 2015

Treatment	*Disease intensity (%)	
	Leaves	**Twigs**
Ampelomyces quisqualis @ 50%+Flusizole 48 EC @ 0.02%	5.70 (13.79)	0.96 (5.61)
Pruning 6 cm (below diseased site) + Flusizole 48 EC @ 0.02%	4.67 (12.46)	0.48 (3.98)
Pruning 6 cm (below diseased site)+ *Ampelomyces quisqualis* @ 50%	9.93 (18.34)	2.88 (9.75)
Pruning 6 cm (below diseased site)+ *Ampelomyces quisqualis* @ 50%+ Flusizole 48 EC @ 0.02%	2.33 (8.76)	0.16 (2.31)
Check	56.40 (48.67)	17.15 (24.44)

CD **(P≤0.05)**

Leaves	Twigs
0.45	0.88

*Mean of three replications

Figures within parenthesis are arc sine transformed values

Chapter-5

DISCUSSION

Euonymus japonicus is an important ornamental plant of aesthetic importance world over and grown all over the Kashmir valley in public as well as private gardens. It is often cultivated in parks and gardens, especially to form hedges. Powdery mildew is probably one of the most common and widely distributed disease of *E. japonicus* (Naseripour *et al*. 2014). The powdery mildew caused by *Oidium euonymi-japonici* causes serious damage to *E. japonicus* by reducing its quality and deteriorating all its decorative and aesthetic values. (Nademjad, 1966).

In the present study, an intensive survey for powdery mildew of *E. japonicus* was carried out in three zones of Kashmir valley *viz*., central, south and north zones during 2012 and 2013 to generate precise information on the distribution and intensity of the disease. The data on survey revealed that the powdery mildew incidence as well as Per cent Disease Intensity (PDI) varied from zone to zone and locality to locality, and was more severe during 2013 than that of 2014. The average disease incidence and PDI varied from 56.67 to 86.70 per cent and 20.89 to 53.33 per cent on leaves and 8.19 to 31.27 and 3.62 to 18.60 per cent on twigs with highest recorded in south zone (78.06 and 48.01 per cent on leaves and 23.59 and13.31 per cent on twigs) followed by central zone (77.00 and 43.43 per cent on leaves and 23.34 and 13.18 per cent on twigs) and least in north zone (73.83 and 40.02% on leaves and 19.16 and 10.54% on twigs). However, the average disease incidence and PDI was recorded maximum in Nishat location of central zone both on leaves (86.70 and 53.33%) and twigs (31.27 and 18.80%) and minimum on both leaves (56.67 and 20.29%) and twigs (8.19 and 3.62%) was recorded in the Iqbal Park location of the same zone. In south zone, the average disease incidence and PDI on both leaves (79.47 and 49.26%) and twigs (28.57 and 17.28%) was found to be maximum at Verinag and minimum at Achabal (76.64 and 46.00% on leaves and 20.31 and 11.08% on twigs). Similarly in north

zone the average disease incidence and PDI was maximum on both leaves (79.25 and 43.11%) and twigs (16.97 and 9.72%) at Tangmarg and minimum at Manasbal (73.83 and 36.80% on leaves and 16.97 and 9.72% on twigs). Such variations in powdery mildew incidence and intensity have been reported by earlier workers (Yarwood, 1954; Palti, 1988; Sharmila, 2001). However, average disease incidence and PDI on leaves was higher during 2013 (76.37 and 45.05%) compared to 2012 (75.14 and 42.60%). The disease incidence and PDI on twigs followed the similar pattern. The higher incidence and intensity of powdery mildew during 2013 might be attributed to the temperature and relative humidity prevailing were favorable for disease development and spread.

The variation in the disease could be attributed to the variations in environmental conditions, planting pattern, build up of inoculum and variety grown across the surveyed areas. The variations in the extent of disease have been attributed to similar factors and are in agreement with the findings of several (Raghavendra, 2005 and Solanki *et al*., 1999).

It was observed that the disease progress under natural field conditions was more during cool and dry weather conditions which coincided with the months of April and May. Almost similar observations have been reported by Yarwood (1954) in case of powdery mildew of different crops.

Disease symptoms started appearing in the second week of April as chlorotic areas on upper leaf surface with small circular to irregular whitish spots of 1.2 to 2.3 mm in size on corresponding lower surface. As the disease progressed, several spots coalesced to form large patches of powdery growth on both the surfaces of leaves and leaves started showing mottled appearance. Subsequently, the disease appeared on twigs and, in severe cases, resulted in witches broom appearance. Leaves curled and got distorted. Thereafter, the leaves withered, shriveled and dried up. Almost similar symptoms had been reported by many workers on *E. japonicus* (Mameth and Chatfield, 2009; Li *et al*., 2011; Williamson, 2013) and on many other ornamental plants such as lisianthus,

nasturtium, lily, impatiens, dogwood and crape (Reis *et al*., 2007; Mameth and Chatfield, 2009).

In addition to survey, the status/prevalence of the powdery mildew on various cultivars and weeds was recorded to investigate the importance of *O. euonymi japonici* infection on these cultivars/plants in providing inocula for one another. The present findings through pathogenicity tests and cross inoculation of *O. euonymi japonici* strains indicated a positive reaction existed among *E. japonicus, E. aureopictus, E. albomarginatus, E. microphyllus, E. fortunii, E. aureaomarginatus*. However, in case of weeds, the cross inoculation study indicated a negative reaction. Furthermore, potential of transmissibility of *O. euonymi japonici* from one Euonymus cultivar to another observed in the present study suggest that the survival of the pathogen on various Euonymus hosts would provide the primary as well as secondary inocula for all these cultivars. Thus necessitates the management of the disease for one cultivar equally essential for the other euonymus host cultivars.

The host range study was conducted to know the ability of *O. euonymi-japonici* to survive on different hosts. This would help to locate source of initial inoculum of pathogen in the disease cycle and also in developing suitable control measures. The transmissibility of *Oidium euonymi-japonici* from one host to another is of great practical interest in assessing the danger of infected weeds, other euonymus species and other hedges causing infections in *E. japonicus*. In the present cross inoculation study, other euonymus species, viz., *E. aureopictus, E. albomarginatus, E. microphyllus, E. fortunii, E. aureaomarginatus,* showed positive reactions whereas, weeds showed negative reaction.

All the available evidence points to the fact that populations of *O. euonymi-japonici* attacking its major host are not markedly specialized, they are able to infect various other species of euonymus within the Celastreace family and vice-versa. The results are in agreement with the findings of Dreistadt *et al*.

(1994) who also reported the *O. euonymi japonici* infection on variegated species of euonymus other than *E. japonicus*.

The morphological studies revealed that the mycelium was branched, septate, hyaline in colour and measuring 3.4 to 7.5 µm (average 5.25 µm) in width. The pathogen was characterized by elliptical conidia measuring 26.3-39.5 ×8.7-12.3 µm (average 33.9×10.8 µm) in length and width, respectively and after germination, form a germ tube with lobed aspersorium. On host, the mycelium was amphigeous, whitish and later on turned whitish grey. The pathogen produced conidia singly or in chains on erect conidiophores. Similar morphological characteristics of the pathogen were given by Li *et al.* (2011).

Healthy leaves of *E. japonicus* were inoculated by rubbing infected twigs on both the surfaces of healthy leaves and also on twigs without creating injury. Symptoms appeared on inoculated leaves first on lower surface and then on upper surface. The lesions were first small whitish irregular spots and measured 0.6 to 1.2mm in diameter. The colour changed to grey and size increased with the passage of time.

Environmental factors decide the epidemic of powdery mildew of *E. japonicus*. The environmental factors like temperature, relative humidity and rainfall are important for disease development and these environmental factors are being used to forecast disease intensity.

During 2013, the powdery mildew symptoms were first observed on 15th standard week when the maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall in the previous week were 20.50 ^{o}C, 5.29 ^{o}C, 65.86 per cent, 5 8.71 per cent and 1.40 mm respectively. The disease intensity increased slowly and reached 56.93 per cent during 26th standard week. The maximum infection rate was observed in the 17th standard week when the maximum temperature, minimum temperature, maximum relative

humidity, minimum relative humidity and rainfall in the previous week were 18.57^{o}C, 8.06 ^{o}C, 86.57 per cent, 63.57 per cent and 3.83 mm respectively.

During 2014, the powdery mildew symptoms were first observed on 16th standard week when the maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall in the previous week was 14.07 ^{o}C, 6.23^{o} C, 82.71 per cent, 76.14 per cent and 7.60 mm respectively. The maximum infection rate of 0.1398 was recorded in 19th standard week when the maximum temperature, minimum temperature, maximum relative humidity, minimum relative humidity and rainfall were 25.21^{o}C, 9.43 ^{o}C, 82.14 per cent, 51.00 per cent and 0.11 mm respectively.

The analysis was made to establish the relationship between weather factors and per cent disease intensity of powdery mildew in highly susceptible variety 'Patens' through correlation and multiple linear regression analysis. The relationship between powdery mildew per cent disease intensity and weather factors during 2013 indicated significant positive correlation between maximum and minimum temperatures and non significant negative correlation with maximum relative humidity, minimum relative humidity and rainfall. During 2014, same correlation matrix was obtained as in 2013. The pooled correlation analysis yielded the similar correlation coefficient except the minimum relative humidity which showed the significant negative correlation with the percent disease intensity. The results are in conformity with Jacob *et al*. (2007) who reported that disease intensity of powdery mildew of tomato caused by *Oidium neolycopersici* was positively correlated with the temperature range from 15 to 25°C. The present results also in agreement with Gupta (1989) who observed that temperature range of 11 to 14°C (minimum) and 17 to 31°C (maximum) along with moderate relative humidity (64 to 72 %) was most congenial for the development of powdery mildew of mango. Rainfall did not play any significant role on appearance and disease development, but dry weather favored the development of the disease. Jat and Goyal (2009) also evaluated that temperature

had significant and humidity had non-significant correlation with disease intensity of powdery mildew of ber incited by *Oidium erysiphoides* f. sp. *ziziphi* and thus are in conformity with our results.

The multiple linear regression equation was fitted to the data and the equation arrived for all the weather parameters during 2013 was, $\hat{Y}_1$ - 122.10 + $3.04X_1 + 2.05X_2 + 0.638X_3 + 0.228X_4 - 1.156X_5$ and for 2014 was, $\hat{Y}_2$= -155.09+ $5.41\ X_1 + 3.767X_2 - 0.772\ X_3 + 1.357\ X_4 + 2.911\ X_5$. The pooled equation of 2013 and 2014 derived was $\hat{Y}$= -174.60+ $4.87X_1 + 1.80\ X_2 + 0.083X_3 + 1.203\ X_4 - 0.086\ X_5$. Hence, the weather factors influence the PDI for 2013 to the extent of 81 per cent and for 2014 to the extent of 94 per cent, and for the pooled years of 2013 and 2014, to the extent of 90 per cent.

In general, comparatively low relative humidities are associated with warm weather, when the powdery mildew pathogens spread most rapidly. The ability of the powdery mildews to spread under dry climatic conditions might be largely due to the capacity of their conidia to germinate at lower humidities (Yarwood, 1952). Guzmann *et al.* (2003) observed that, temperature 32^{o}C and above, very low RH and continuous exposure to higher relative humidity reduced spore germination of disease progress of powdery mildew of tomato and thus are in agreement with our results.

The present investigation regarding the reaction of 6 cultivars of *E. japonicus* to *O. euonymi-japonici* revealed that the cultivar screened show different reactions against the pathogen under artificial inoculated conditions. The average PDI in the test cultivars ranged from 1.40 in cultivar Silver queen to 58.40 in cultivar Patens. On the basis of disease reaction the cultivars were categorized as moderately resistant (Emalard Giety, Canadale Gold and Box-leaf euonymus), resistant (Silver King and Silver Queen) and susceptible (Patens). The study is in agreement with many workers who reported that green cultivar Patens is very susceptible and variegated varieties of euonymus like aureomarginatus,

albomarginatus, aureopictus are more resistant than nonvariegated types like patens (Pscheidt and Ocamb 2015; Van, 2008; Gubler1992)

Observations regarding the perpetuation studies reveal that the fungus may overwinter on the intact twigs and leaves in the form of dormant mycelium and with the onset of spring the fungus produces conidia which are splashed by rains and lodged on leaf surfaces thus causing fresh infection. Similar observations were recorded by Salmon (1905) who observed that fungus *O. euonymi-japonici* overwinters as mycelia on leaves. The results are also in agreement with Horst (1990) and Van (2008) who observed that the powdery mildew fungi *O. euonymi-japonici*survive from season to season on infected twigs and leaves of *E. japonicus*.

Four sprays of fungitoxicants viz., flusilazole, dinocap, wettable sulphur, $KHCO_3$, propiconazole, triadimefon, myclobutanil, difenconazole, hexaconazole and flusilazole at their recommended doses and concentrations were given with the first spray immediately after the first appearance of disease.

In the present investigation, it is evident that all fungitoxicants under study were found to be effective in controlling the powdery mildew of *E. japonicus*. Among these, flusilazole 40EC @0.02% was most effective in controlling the disease followed by triadimefon 25 WP@ 0.05%, difenconazole 25 EC @ 0.03%, propiconazole 25 EC @0.03%, myclobutanil 10WP @0.03%, dinocap 48 EC @ hexaconazole5EC @ 0.03%, wettable sulphur 80 WP@ 0.2% and $KHCO_3$. These findings are in agreement with the results of Williamson (2013).

The results are also in agreement with the findings of Dhruj *et al*. (2000) who reported that propiconazole, hexaconazole, triadimefon, dinocap and sulphur gave significant control powdery mildew of fenugreek.

Similar results were also obtained by Van (2008) who reported that elemental sulfur, propiconazole, triadimefon and myclobutanil provided excellent control powdery mildew of *E. japonicus*.

The results showed that $KHCO_3$ was effective against the powdery mildew of *E. japonicus.* The results are in agreement with Ziv and Hagiladi (1993) who observed that application of aqueous solution of sodium or potassium bicarbonate along with horticultural oil to plant foliage effectively controlled powdery mildew on *E. japonicus*.

The results are also in agreement with Hartman (2008) who reported the control of powdery mildew of landscape ornamentals like euonymus and dogwood by spraying triadimefon, propiconazole, myclobutanil and potassium bicarbonate.

The present study was designed to reduce chemicals in agricultural processes and find out the most suitable non-chemical method to protect the Euonymus against the powdery mildew. As the powdery mildew limits both the aesthetic and ornamental importance of Euonymus, disease control strategies demand the extension of mechanical disease control and genomic approaches. The principal mechanisms involved include mycoparasitsm, antibiosis and induced resistance. Biological control method of disease control seems to be safe.

The culture filtrates of bioagents reduced the PDI of powdery mildew of *E. japonicus* less as compared to chemical fungicides. Among the culture filtrates of bioagents tested *A. quisqualis* was most effective in reducing the disease intensity followed by *P. fluorescens* and *T. viride*. The present findings are in agreement with Biju (2000) who reported the ineffectiveness of bioagents over the fungicides in controlling powdery mildew peas.

The low effectiveness of the bioagents might be attributed to unfavorable environmental conditions prevailing in the field for the establishment of bioagent (Belanger *et al*., 1994). Though, Roger (1959) reported that, many members of Erysiphaceae are parasitized by hyperparasites, Yarwood (1957) considered that, control of mildews using hyperparasites is unlikely to be successful because the host fungus and the parasite are favoured by different weather.

The results obtained are in conformity with Gu and Ku (1997) who reported that the most important hyperparasite for powdery mildew of *E. japonicus* is *Ampelomyces quisqualis.* Ravikumar (1998) also found that, different biocontrol agents *Trichoderma viride*, *P. fluorescens* and *Gliocladium virens* were less effective against powdery mildew of rose both in laboratory and field conditions compared with fungicides and is in conformity with our results.

Our findings are also in conformity with Romero *et al.* (2007) who found that most explored agents for biocontrol of powdery mildew in cucumber is a mycoparasite *A. quisqualis*. Pawar (2015) also reported that pre inoculation spray of *T. viride* and *A. quisqualis* gave excellent control of powdery mildew of pumpkin.

The efficacy of the culture filtrates of the tested microbial isolates against powdery mildew might be due to production of antifungal substances like gliotoxin and viridin. These substances stimulate plant resistance and also stimulate the plants to produce the metabolites and growth promoters such as indols which increased the plant resistance against the powdery mildew.

Pruning the plants 6 cm below the disease site proved to be most effective in controlling the disease intensity of powdery mildew of *E. japonicus*. The results are in agreement with those of Fraedric (1999) and Hansen (2009) who reported that sanitation practices like pruning the plants helps in reducing the intensity of powdery mildew of ornamentals.

An integrated disease management practice was devised to protect the *E. japonicus* plants against the powdery mildew. Pruning the plants 6 m below the disease site with alternate sprays of *A. quisqualis* @ 50% and flusilizole 48 EC @ 0.02% proved to be most effective in controlling the disease intensity. Future work will concentrate on alternating effective fungicides with flusilazole would be an important aspect to prevent the resistance development by pathogen to flusilazole when used alone and repetitively in the management of powdery

mildew. Further studies are required to concentrate on reducing the pesticides and adapt an eco-friendly approach for the management of the disease. The results are in conformity with LaMondia (2001) and Olson (1998).

The present findings indicate that powdery mildew is present throughout the Kashmir on *E. japonicus* and other Euonymus species though in varying proportions. The infected species could provide inocula for one another. The results further revealed that the disease could be effectively controlled by employing integrated strategies comprising of pruning (6cm below diseased site), biological and chemical interventions. The biological and chemical interventions comprised of two applications of *A. quisqualis* (@ 50%) and flusilazole (@ 0.02%) alternatively at an interval of 15 days.

Chapter-6

SUMMARY AND CONCLUSION

The present investigation entitled "Studies on Powdery Mildew (*Erysiphe euonymi-japonici* Veinn. Bourg.) of Euonymus (*Euonymus japonicus* Thunb.) in Kashmir valley" was carried out at Division of Plant Pathology SKUAST-Kashmir, Shalimar campus Srinagar during 2012 to 2015. In addition, survey of Euonymus growing areas of the valley was also conducted to ascertain the status of the disease. The results obtained are summarized here under.

The survey of central, south and north zones of Kashmir revealed that powdery mildew of euonymus was prevalent in all the 16 locations with overall disease incidence of 76.37 (56.67 to 86.70%) and 22.03 (8.19 to 31.27%) per cent and overall per cent disease intensity (PDI) of 43.82 (20.89 to 53.33%) and 12.34 (3.62 to 18.80%) on leaves and twigs, respectively. The highest average disease incidence (78.06% on leaves and 23.59% on twigs) and highest PDI (48.01% on leaves and 13.31% on twigs) was observed in south zone. Least average disease incidence (73.83% on leaves and 19.16% on twigs) was observed in north zone. The highest average per cent disease intensity (PDI) on leaves (48.01%) and twigs (13.31%) was observed in south zone. Least average PDI (40.02% on leaves and 10.54% on twigs) was observed in north zone. Amongst the locations, highest average disease incidence and PDI on both leaves (86.70 and 53.33%) and twigs (31.27% and 18.80%) was observed in Nishat, while as the lowest average disease incidence and PDI on leaves (56.67 and 20.89%) and twigs (8.19 and 3.62%) was recorded at Iqbal Park of central zone.

Under natural conditions, the disease first appeared in the 2nd week of April as chlorotic spots on the upper surface of the foliage, while as its corresponding lower surface was covered with whitish circular to irregular spots measuring about 1.8 mm. Subsequently whitish powdery mass appeared on both the leaf surfaces. Periodical changes in shape, size and color of the spots were

noticed and finally whitish to whitish grey powdery mass covered the whole leaf surface and also symptoms appeared on twigs. The leaves got mottled, curled, distorted, withered and shriveled.

On the basis of morphological characters and comparing them with the standard manual as well as pathogenicity tests, the pathogen was identified as *Oidium euonymi-japonici* Acrang. (Sacc.)

The morphological studies of the pathogen in laboratory revealed that mycelium was septate with the average diameter of 5.25 µm and produced elliptical conidia on long and unbranched conidiophores (91.5 ×9.1 µm). Conidia were single celled, hyaline, and borne either singly or in chains. The average size of conidia was found to be 26.3-39.5×8.7-12.3.

The studies on effect of weather factors on development of disease revealed that, 16^{th}, 17^{th}, and 18^{th} standard weeks (20^{th} April to 4^{th} May) were highly favorable for development of disease. The correlation study revealed that maximum and minimum temperature showed significant positive correlation with the disease development with correlation coefficients (r) of 0.914 and 0.91, respectively, whereas minimum relative humidity was showed significant negative correlation (-0.664). The maximum relative humidity and rainfall was negatively correlated with the disease development with r values equal to -0.434 and -0.364, respectively.

The multiple linear regression equation was fitted to the data and the equation arrived for all the weather parameters as $\hat{Y}= -174.60+ 4.87X_1 +1.80\ X_2 +0.083X_3 + 1.203\ X_4 - 0.086\ X_5$

Where, X_1 = Maximum temperature (^oC), X_2 = Minimum temperature (^oC), X_3 = Relative humidity (%) maximum, X_4 = Relative humidity (%) minimum and X_5 = Rainfall (mm).

The present study established that pathogen overwintered as mycelium on twigs and leaves which started producing conidia in 3^{rd} week of February. The

maximum production and viability of conidia on intact leaves was observed to be $4.4 \times 10^4/cm^2$ leaf area and 83.40 per cent, respectively during the first week of April. No conidia and mycelium was observed on the leaves and twigs buried 10 cm deep in the soil.

The perpetuation/host range study further indicated that more or less similar powdery mildew symptoms also occurred on *Euonymus aureopictus, Euonymus albomarginatus, Euonymus microphyllus*, *Euonymus fortunii* and *Euonymus aureaomarginatus*. The inocula from these euonymus species and *E. japonicus* were separately cross inoculated with one another as well as cross inoculated with weeds *viz*. *Trifolium repens, Plantago major, P. minor, Ranunculus acris, Convolvulus arvensis, Cynodon dactylon, Rosa indica* and *Ligustrum ovulifolium* and vice versa. The findings indicated that inoculum from *E. japonicus* successfully infected all the Euonymus genotyps viz., *E. aureopictus, E. albomarginatus, E. microphyllus*, *E. fortunii* and *E. aureaomarginatus* and vice-versa but failed to infect the weeds and other test hosts.

During the course of present study, all the nine fungitoxicants (at recommended concentrations) were significantly effective in controlling the disease compared to check under natural field conditions. Flusilazole 40 EC (@ 0.02%) proved to be significantly most superior in reducing the disease by exhibiting PDI of 8.80 and 1.36 on leaves and twigs and was followed by triadimefon 25 WP (@0.05%) and difenconazole 25 EC (@0.03%) which recorded PDI of 9.76 and 10.06 on leaves and 2.00 and 2.21 on twigs, respectively as compared to unsprayed check (58.80% and 18.05%) whereas potassium bicarbonate ($KHCO_3$) proved to be least effective (21.43% and 4.57%).

Cultural filtrates of the three test biocontrol agents viz., *T. viride*, *Pseudomonas fluorescens* and *A. quisqualis* (@25 and 50%) as foliar applications significantly reduced the powdery mildew of *E. japonicus* under natural conditions of disease development. *A. quisqualis* proved to be significantly superior in controlling the disease by exhibiting PDI of 34.71 and 9.72 on leaves

and twigs as compared to 58.16 and 17.10 recorded in check, respectively. *T. viride and P. fluorescens* differed statistically from each other in reducing the disease by exhibiting PDI of 40.53 and 39.45 on leaves intensity and on leaves and 12.17 and 11.70 on twigs, respectively.

The various levels of pruning viz., 2, 4 and 6 cm below the diseased region tested in present study, significantly checked the disease compared to unpruned check during both the years of 2013 and 2014. Pruning of twigs 6cm below the diseased site, proved significantly superior to other pruning levels by exhibiting the PDI 18.79 and 5.08 on leaves and twigs as against 57.99 and 17.88 recorded in the check, respectively. It was observed that 4 and 2cm pruning levels recorded PDI of 27.52 and 36.20 on leaves and 9.11 and 11.99 on twigs, respectively.

The integrated management of powdery mildew of *E. japonicus* indicated that pruning of Euonymus 6cm below the mildewed area along with four foliar sprays of *A. quisqualis* (@ 50%) and flusilazole 40 EC (@ 0.02%) applied alternatively at 15 days interval proved to be most effective in restricting the PDI as low as 2.33 and 0.16 on leaves and twigs compared to 56.40 and 17.15 recorded in the untreated check, respectively.

It was also observed that among the six cultivars screened under artificial inoculated conditions, variegated cultivars viz., Canadale Gold, Emerald Gaiety and Box leaf euonymus exhibited 13.86, 17.59 and 10.92 PDI and were categorized as moderately resistant to the disease. The cultivars Silver King and Silver Queen exhibited PDI of 2.49 and 1.40 and were categorizes as resistant whereas, the cultivar Patens exhibited the PDI of 58.40 and was categorized as susceptible to the disease.

From the present study, it can be concluded that powdery mildew is an important foliar disease of *E. japonicus* in the valley and reduces its aesthetic and ornamental value. The disease was initiated by *O. euonymi-japonici* in 2nd week of

April and disease progress was recorded maximum during the months of April and May. The present investigation revealed that powdery mildew of *E. japonicus* can be effectively managed through integrated approach comprising of four foliar sprays of *A. quisqualis* (@ 50%) and flusilazole 40 EC (@ 0.02%) applied alternatively at 15 days interval as well as pruning of twigs atleast 6cm below the diseased area/portion. In addition, variegated cultivars such as Canadale Gold, Emerald Gaiety, Box leaf euonymus Silver King and Silver Queen exhibited 13.86, 17.59, 10.92, 2.49 and 1.40 PDI proved to be moderately resistant to resistant against the powdery mildew pathogen and possess the potential for evolving resistant genotypes through breeding for resistance.

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*Original not seen.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. Vinay Sagar, Senior Scientist, Division of Plant Pathology, ICAR, CPRI, Shimla, during viva-voce Examination held on 8th June 2016 have been incorporated in the manuscript **"Studies on Powdery Mildew (*Erysiphe euonymi-japonici* Veinn-Bourg.) of Euonymus (*Euonymus japonicus* Thunb.) in Kashmir valley"** submitted by **Mr. Rayees Ahmad Ahanger, Ph.D. student (Regd. No. 2011-363-D) of Plant Pathology discipline.**

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