EPIDEMIOLOGY AND MANAGEMENT OF STEMPHYLIUM BLIGHT OF GARLIC

Thesis

by

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CERTIFICATE-I

This is to certify that the thesis titled**, "Epidemiology and management of Stemphylium blight of garlic",** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY** in the discipline of **PLANT PROTECTION** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) – 173230 is a bonafide research work carried out by **Ms. Chidembra Bhardwaj (H-2016-72-M)** daughter of Shri Ashwani Kumar Bhardwaj under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

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

Place: Nauni-Solan
Dated:

(Dr Sandeep Kansal)
Major Advisor

CERTIFICATE-II

This is to certify that the thesis titled, **"Epidemiology and management of Stemphylium blight of garlic"**, submitted by **Ms. Chidembra Bhardwaj (H-2016-72-M)** daughter of Shri Ashwani Kumar Bhardwaj to the Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) – 173230, India in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE (AGRICULTURE) PLANT PATHOLOGY** in the discipline of **PLANT PROTECTION** has been approved by the Student's Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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I owe entire responsibility for all the errors and omissions

Place: Nauni, Solan
Dated: **Chidembra Bhardwaj**

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LIST OF ABBREVIATIONS

@	=	At the rate
%	=	Per cent
/	=	Per
<	=	Less than
>	=	More than
µl	=	Microlitre
µm	=	Micrometer
ANOVA	=	Analysis of Variance
C.D.	=	Critical Difference
CFU	=	Colony Forming Unit
cm	=	Centimeter
CRD	=	Completely randomized design
cv.	=	Cultivar
cvs.	=	Cultivars
dia.	=	Diameter
EC	=	Emulsified concentrate
et al.	=	and co-workers
h	=	Hours
ha	=	Hectare
i.e.	=	That is
ICBR	=	Incremental Cost Benefit Ratio
kg	=	Kilograms
m	=	Meter
mg	=	Milligram
ml	=	Millilitre
mm	=	Millimeter
^{o}C	=	Degree centigrade
PDA	=	Potato Dextrose Agar
ppm	=	Parts per million
q	=	quintal
RBD	=	Randomized block design
SC	=	Soluble concentrate
SG	=	Solan Garlic
sp.	=	species
viz.	=	Namely
var.	=	Variety
WG	=	Wettable granule
WP	=	Wettable powder

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Chapter 1

INTRODUCTION

Garlic is the second most important bulb crop grown throughout India. It is commonly termed as "*Lasan*", belongs to family Amaryllidaceae and botanically known as *Allium sativum* (Linnaeus). It is widely cultivated in India for home consumption and for earning foreign exchange as well.

It is a herbaceous annual. The inflorescence of garlic is known as 'scap'. The economic yield is obtained from an underground developed part known as bulb. The garlic bulb is a multiple or compound bulb consisted of small bulbels or bulblets or segments called as cloves i.e. 10 to 20 in number and they are covered with a white or pinkish parchment like membrane or sheath.

Original abode of garlic is said to be Central Asia and Southern Europe especially Mediterranean region (Thompson & Kelly 1957). It is a perennial plant, producing narrow flat leaves which are milder in flavor than the bulbs, and are most often consumed when immature and tender. Garlic leaves are a popular vegetable in many parts of Asia. It is widely used around the world for its pungent flavor as a seasoning or condiment ingredient. It is chiefly used for flavorings and seasoning vegetable dishes. This crop is one of the important foreign exchange earner crops of India because of the good quality and quantity of garlic exported every year from the country.

Garlic cloves are used for consumption (raw or cooked) or for medicinal purposes. It is a rich source of protein, phosphorus, calcium, magnesium, potash and ascorbic acid. One fresh peeled garlic clove has 62.8% moisture, 6.3% protein, 0.1% fat, 0.8% fiber, 29.0% carbohydrates, 0.03% calcium, 0.31% phosphorus etc. (Srivastava & Singh 1977). Beside these, it is also well known for having numerous valuable medicinal properties. The garlic extract has insecticidal, fungicidal and bactericidal action. The garlic juice is given in pulmonary tuberculosis, rheumatism, sterility, impotency, cough and red eyes (Pruthi, 1979). Garlic can inhibit reproduction of toxic germs in the stomach. It stops synthesis of a carcinogen named nitrosamine (Singh and Pandey, 1989). It also reduces blood sugar (Brahmachari and Augusti, 1962).

Garlic is grown globally but China is leading country in area and production followed by India, Republic of Korea, Egypt and Russian Federation as per FAO. India beholds the second position in world's total area and production under garlic. In India it is cultivated in an area of 2.47 lakh hectares with the production of 12.59 lakh tonnes and productivity of 5.09 tonnes per ha (Anonymous, 2015).

In India major Garlic producing states are Madhya Pradesh tops in area 92.50 thousand hectares, followed by Rajasthan 107.97 thousand hectares and Gujarat 10.10 thousand hectares (Anonymous, NHRDF, 2016-17). Although, the production and productivity is quite low in Himachal Pradesh as compared to other states but in the past one decade. In Himachal Pradesh has emerged as a potential garlic producing state with acreage of about 4430 hectares with production of 7720 tonnes and productivity of 1.74 tonnes/ha (Anonymous, NHRDF, 2016-17). However, successful cultivation of garlic is consistently threatened by various diseases, amongst which Stemphylium blight caused by *Stemphylium vesicarium* is an important disease of garlic causing significant yield loss. The disease was first recorded on Allium in India by Rao and Pavagi in 1975. The outbreak of Stemphylium leaf blight occurs every year in a moderate to severe form in garlic growing districts of Himachal Pradesh.

The disease appears in severe form after a hailstorm occurring during humid spring season. The initial symptoms of disease are characterized as small, irregular to oval, white flecks surrounded by a bright yellow margin which enlarged to produce zonate lesions, light to tan brown in colour. These lesions expanded, merged and coalesced resulting in withering of leaf tips and blightening of the entire foliage. The unusual rise in temperature during cropping season from normal, coupled with intermittent rains leads to epidemic development of disease. The disease resulted in 30% yield losses on an average and the losses may be as high as 70 per cent.

Though, Stemphylium blight is an important foliar disease yet little study on this disease has been conducted so far. Since, it is an important limiting factor for garlic production, comprehensive studies on etiology, epidemiology and management are essential. Hence, the present investigation is being taken up with the following objectives:-

i. To study the magnitude of Stemphylium blight of garlic in different growing localities of Solan and Sirmour districts of Himachal Pradesh.

ii. To study the etiology of the disease.

iii. To study the role of abiotic factors on disease development.

iv. To evaluate the efficacy of fungicides against the disease.

Chapter 2

REVIEW OF LITERATURE

The genus *Stemphylium* may be defined as hyphomycetous anamorphs characterized by pigmented, muriform conidia that develop at a very restricted site in the apex of distinctive conidiophores (Simmons, 2007). *Stemphylium vesicarium,* the causative organism of Stemphylium blight of garlic has several reported synonyms as appended here under :-

Stemphylium vesicarium (Wallr.) E. Simmons

Perfect state: *Pleospora allii* (Rabenh.) Ces. & De Not.

Helminthosporium vesicarium Wallr. (1833).

Macrosporium vesicarium (Wallr.) Sacc. (1886).

Pleospora allii (Rabenh.) Ces. and De not., (1863).

Sphaeria allii Rabenh., (1846).

2.1 GEOGRAPHICAL DISTRIBUTION

Stemphylium leaf blight disease of garlic caused by *Stemphylium vesicarium* (Wallr.) E. Simmons and its perfect state *Pleospora allii* was reported for the first time in the world in 1935 from Burma by Su as *Macrosporium parasiticum* (*Pleospora herbarum*). In India Stemphylium leaf blight on garlic was reported for the first time in 1975 by Rao and Pavgi. They observed that the infection remain confined to the leaves and inflorescence stalks.

Stemphylium vesicarium has been reported on several hosts from several countries as on Lucerne from South Africa (Lamprecht *et al*., 1984), on onion from Portugal (Tomaz and Lima 1986), on asparagus from Washington state (Johnson, 1987) and South Africa (Thompson and Uys 1992), on pears from Italy (Brunelli and Penti 1989) and Spain (Baroja, 1999), on coconut from Southern Oman (Hammouda, 1991), on *Allium* spp. (Cho and Yu 1998) from Korea and on garlic from South Africa (Aveling and Naude 1992), Brazil (Boiteux *et al*., 1994), Spain (Basallote *et al*., 1999) and Australia (Suheri and Price 2000b). The causal agent of severe foliar blight on

garlic (*Allium sativum*) and on onion (*Allium cepa*) at the Rangel and Sucre municipalities of Merida State, Venezuela, was identified as *Stemphylium vesicarium* and this was the first report of *S. vesicarium* causing severe leaf blight disease on garlic and onion in Venezuela (Cedeno *et al*., 2003).

In India, *Stemphylium vesicarium* was reported on onion from Maharashtra (Patil and Patil, 1992) and from Bihar (Sinha *et al.,* 1995). Sinha *et al.* (1998) observed the anamorph *Stemphylium vesicarium* and teleomorph *Pleospora* sp. on garlic as a new report from India. The incidence of leaf blight on garlic has also been reported from Himachal Pradesh (Sugha and Kumar 2005). Shubana *et al.* (2008) have observed for the first time the Stemphylium blight of onion pathogen *S. vesicarium* (Wallr.) E. Simmons, perfect state; *Pleospora allii* (Pers. ex. Fr.) Rabenh. from Jammu and Kashmir.

2.3 SURVEY AND SURVEILLANCE

The survey and surveillance studies conducted by Gupta and Pandey (1986) revealed the prevalence of Stemphylium blight of onion throughout the country and observed the higher yield losses in northern India.

Bakr (1993) recorded significant (62%) reduction in the yield of lentil which was found to be associated with Stemphylium blight caused by *Stemphylium* sp.

The survey studies conducted by Suheri and Price (2000b) indicated 100% disease incidence on three commercial garlic cultivars grown at Waikeries, Australia, while the Stemphylium blight severity ranged between 10-30 per cent one week before harvest of the crop.

Sugha and Suman (2005) have reported that the incidence of leaf blight in garlic during a study in February, March and April 2003 at 103 localities (found in Bilaspur, Chamba, Hamirpur, Kangra, Kullu, Mandi, Shimla, Sirmaur, Solan and Una districts) in Himachal Pradesh. The average disease severity ranged from 37.8 (Shimla) to 65.5% (Kullu).

Zheng Lu *et al*. (2009) reported the yield losses as high as 70% with an average yield loss of 30% due to Stemphylium blight of garlic in garlic in Dangyang County of China.

Efath Shahnaz *et al.* (2018) observed that *Stemphylium vesicarium* was invariably found associated with typically blighted onion leaves throughout the growing period (82.75% in January to 77.40% on February), though its relative occurrence declined slightly in March (49.15%) and April (37.75%).

2.4 SYMPTOMATOLOGY

Rao and Pavagi (1975) gave a comprehensive description of symptoms of Stemphylium blight of onion and garlic. According to them, the infection appeared as small, yellow to pale orange flecks or streaks in the middle of the leaf. These soon developed into elongated, spindle-shaped to ovate-elongated spots, often reaching the leaf tips and becoming surrounded by a characteristic pinkish margin. They further observed that these elongated spots turned grey at the centre, which became brown to dark olive-brown with the development of conidiophores and conidia of the pathogen. The spots frequently coalesce into extended patches, blighting the leaves and gradually the entire foliage.

Boiteux *et al*. (1994) observed disease symptoms consisting of elliptical, dark-brown to black, zonated leaf spots 0.5 to 4.0cm long by 0.5 to 2.5 cm wide. The lesions eventually coalesced and killed the older leaves.

Hassan *et al*. (2007) have reported that in onion that Stemphylium leaf blight symptoms on the leaves and seed-stalk started with tip necrosis followed by small white and/or large purple spots.

Zheng *et al*. (2009) have reported a new leaf blight disease (*Stemphylium solani*) on garlic manifesting high severity in China. According to them, the initial symptoms consisted of multiple, small, irregular to oval, white leaf spots, which later enlarged to produce sunken purple lesions, sometimes surrounded by a bright yellow margin. With the disease progression, the lesions expanded and merged, resulting in the withering of leaf tips.

Polat *et al.* (2012) have studied the leaf blight caused by *S. vesicarium* on garlic in Turkey. They observed initial symptoms as white flecks that enlarged and produced sunken purple lesions, sometimes surrounded by a yellow to pale brown border.

Koike *et al*. (2013) have described the symptoms caused by *S. vesicarium* on commercial parsley in Coastal (Ventura County) California as circular to oval, tan to brown leaf spots, resulting in the loss of quality and yield of the crop.

B. Hanse *et al*. (2015) observed the symptoms of Stemphylium blight on Sugar beet (*Beta vulgaris* L.) leaves as small, irregular, yellow spots which subsequently the yellow spots became necrotic and over the entire foliage causing death of leaf tissues.

Katoch and Kumar (2016) described symptoms of Stemphylium leaf blight of garlic as white small oval lesions which later became sunken with a purple colour surrounded by a whitish margin and ultimately extensive necrosis followed by pre-mature desiccation of the plants.

Efath Shahnaz *et al*. (2018) reported that the inoculations of onion plants made with *Stemphylium vesicarium* manifested into small, white, spindle-like lesions which turned to light yellow in colour and later changed to brown or purple. Within few days, the entire leaf gave a blighted appearance.

2.5 PATHOGENICITY

Aveling and Naude (1993) reported pathogenicity on garlic plants (cv. Large Egyptian White) by spray inoculating the garlic leaves to run-off with conidial suspension at a concentration of $5x10^4$ conidia ml^{-1}. The inoculated plants incubated in a mist chamber for 6, 12, 24 or 48 h exhibited similar symptoms as were observed under field conditions. Culture of *Stemphylium vesicarium* reisolated from inoculated plants was found identical to the original cultures, proving Koch's postulates.

Basallote *et al*. (1993) proved pathogenicity of 11 isolates of *S. vesicarium* by spraying garlic and onion plants with conidial suspensions of the fungus, followed by incubation at 18-22°C in a saturated atmosphere for 48 hr. After inoculation with different isolates similar lesion types were reproduced after 5-8 days in both plant species.

Boiteux *et al.* (1994) conducted pathogenicity test of *Stemphylium vesicarium* on 3 week old garlic plants inoculated with conidial suspension $5x10^4$ conidial/ml. The inoculated plants including the control plants were covered with transparent polythene bags for 2 days (temperature range 28-30°C), and then transferred to a greenhouse at 22-26°C. The inoculated plants exhibited characteristic symptom of the disease after 8-

10 days of inoculation and the re-isolated pathogen from these plants was confirmed as *Stemphylium vesicarium* (Wallr.) E. Simmons.

Basallote *et al.* (1999) conducted pathogenicity tests on garlic using isolates of *S. vesicarium* from garlic, onion and asparagus which reproduced symptoms as apical necrosis and white spots within 4-6 days of inoculation, and approximately one week later, individual purple spots developed on the older leaves of some plants. Re-isolations from these infected tissues gave results similar to those from the naturally infected plants.

Hassan *et al*. (2007) confirmed pathogenicity of 15 isolates of *Stemphylium vesicarium* of onion. Inocula were prepared by growing isolates on potato dextrose agar at 27°C for 15 days. The conidial suspension prepared by adding sterile distilled water from each isolate (5×10^4 CFU per ml) was used for spray inoculation of 12 onion plants (110 day old cv. Giza 6), using an atomizer to spray leaves and seed-stalks. After inoculation, plants were covered with polyethylene bags for 48 hours and thereafter the bags were removed and plants were kept under field conditions until symptoms appeared. The inoculated plants revealed the symptoms similar to those observed in onion plants in commercial fields. The fungus was re-isolated from lesions of inoculated plants and was confirmed as *Stemphylium vesicarium*.

Zheng *et al*. (2008) conducted pathogenicity tests of *S. vesicarium* on 2 week old garlic plants by spraying a conidial suspension ($1x10^6$ conidia/ml) containing 0.1% Tween-20 until runoff. Plants were incubated in growth chamber at 25°C and 90% relative humidity. White spots were observed on inoculated leaves after 5 days of inoculation. Koch's postulates were fulfilled by re-isolating *Stemphylium solani* from diseased leaves.

2.6 MORPHOLOGY

Descriptive information on *S. vesicarium* has been presented by Simmons (1969). According to him, the conidiophores were either produced radially from a basal mass of compressed or rounded cells or compactly in palisade from a common basal layer of cells. Conidiophores appeared straight to variously curved, simple or occasionally branched, cylindrical but enlarged apically to the site of conidium production. Condiophores were dilute yellow brown or olive brown (darkening to medium golden brown in the swollen apex), smooth or distinctly flared to 7-9 μm in

diameter, apical sporiferous cells with a single pore 4-7 µm in diameter having as many as five apical proliferations. He further described the morphology of conidia having shape as oblong or broadly oval, sometimes unilateral with 1-5 transverse and 1-2 complete or nearly complete series of longitudinal septa, constricted at one or more places (commonly three of the major transverse septa), dilute or medium golden brown to olive brown, measuring 12-22 x 25-42 µm with an average 17.7 x 33.4 µm i.e., length to width ratio of 1.5-2.7 with a conspicuous basal scar like zone up to 7 µm diameter surrounding a small pore.

Boiteux *et al*. (1994) described morphological characters of *S. vesicarium* as conidiophores of the genus were observed to bear clearly defined vesicular swellings in the apices of the conidiogenous cells which were typically monoblastic and predominantly clavate. Conidia were solitary, dry, rounded at the apex, mild black to olivaceous brown, broadly ellipsoidal, often constricted at three major transverse septa with cell wall minutely verrucose. The number of transverse and longitudinal septa ranged from 1 to 5 and 1 to 3, respectively. The number of lateral constrictions varied from 1 to 4. Average dimensions of 100 conidia obtained directly from the host were 11(8-16) x 26(19-39) µm (length:width ratio 2:4) to 11(7-16) x 27(20-41) µm (l:w ratio 2:5) in PDA culture. The teleomorph (*Pleospora* sp.) was frequently found in culture but never on the host tissue.

Aveling and Rong (1994) studied the conidium formation in *S. vesicarium* through electron microscopy. They observed conidiophores as straight or flexuous, simple, smooth and cylindrical but enlarged apically at the site of conidium production. Smooth and round bud like conidia was observed to produce singly at the apex of the verrucose conidiophores.

Conidiophores arising from stroma varied in number and were observed cylindrical, unbranched, light brown, with enlarged dark brown apical cells and proliferating frequently by the production of new conidiophores from the apices of old ones. Mature conidia were brown, oblong to oval, 25–42 × 12–19 µm, 1–3 transverse constrictions, 1–3 (and upto 4) complete series of longitudinal septa and a variable number of transverse septa (Basallote *et al*., 1999).

Belisario *et al.* (2008) have identified *Stemphylium vesicarium* based on the morphological characters of conidia and conidiophores. They have described the

conidia as golden brown to dark drown, oblong to oval with one to four transverse and one to three longitudinal septa with constriction at one to three of the major transverse septa. Conidial dimensions ranged from 12 to 22×30 to 40 µm. According to them, conidiophores were observed as straight or occasionally with one branch having a swollen apex and one to four septation.

Polat *et al*. (2012) described colonies of *Stemphylium vesicarium* (Wallr.) E. Simmons as effuse, olivaceous brown to black, somewhat velvety; conidia pale to mid-brown or olivaceous brown, verrucose, with up to six transverse and several longitudinal septa, mostly constricted at the major transverse septa, 20-50 x 15-26 µm.

2.7 EPIDEMIOLOGY

Shishkoff and Lorbeer (1989) while studying the effect of leaf wetness duration on disease progression of Stemphylium leaf blight of onion reported that longer the plants were left in a mist chamber after inoculation with the pathogen, the greater the number of lesions per centimeter of leaf. Disease occurred after 18-24 h of exposure to moisture after inoculation. However, abrasion of the leaf surface increased disease severity as rubbed leaves had significantly more lesions per centimeter than non-rubbed leaves.

Montesinos *et al*. (1995) observed the effect of wetness duration and different levels of temperature on disease development of Stemphylium blight on detached leaf and fruit of pear. For both fruit and leaves, there was a rapid increase in severity with increased wetness duration at temperatures of 15, 20, 25 and 30°C. At 5°C, no significant ($P>0.05$) increase of infection levels was observed with increased wetness duration. Maximum disease severity was found at 20 to 25°C with between 18 and 24 h of wetness duration. A significant ($P<0.05$) increase in disease above the background was detected only after 6 h of wetness at 10 to 30°C. In overall the disease severity ranged from 0 to 14.6 mean lesions per fruit and from 0 to 3.5 mean lesions per leaf.

Weather parameters have a pivotal role in the development of foliar diseases. Prados *et al.* (1998) while studying the epidemiology of Stemphylium leaf blight of garlic reported that pseudothecia of the teleomorph (*Pleospora allii*) developed during winter on infected plant debris which consisted the main inoculum source for the initiation of leaf blight disease. The study further indicated that period required for pseudothecial maturation of *Pleospora allii* varied between 1-4 months, depending on

climatic conditions, which was found to be highly correlated with accumulated rainfall and with the period (h) with temperatures between 4.5 and 10.5°C and relative humidity (RH) over 98%. Ascospore release was found to occur between late January and late April.

Basallote *et al*. (1999) studied the effect of leaf wetness duration on the disease incidence and severity of Stemphylium leaf blight in garlic plants under controlled conditions. The disease incidence increased from 60 to 100 per cent with the increase in leaf wetness from 0 h to 72 h. Disease severity (DS) was found to be low and similar for plants of 3 and 5 weeks old at leaf wetness (LW) duration of <72 h and increased exponentially as LW increased beyond 12 h till 48 h. The wetness period longer than 24 h were required for symptom development under controlled conditions.

Suheri and Price (2000a) while investigating the infection of onion by *Stemphylium vesicarium* under a range of controlled temperatures (4-25°C) and leaf wetness periods (0-24 h) observed the infection of onion leaves occurred after 16 h of leaf wetness at 5°C and after 8 h of leaf wetness at 10-25°C. The infection was found to increase with increasing leaf wetness duration up to 24 h at all temperature levels studied.

The epidemiological studies conducted by Suheri and Price (2000b) under field conditions revealed that the periods of higher precipitation for at least 3 days (>6 h leaf wetness/day) alternated with warm dry days (average temperature 20-26°C) favored the rapid development of Stemphylium blight of garlic.

Llorente and Montesinos (2002) determined the effect of interruption of 24 h wetness periods by dry periods of high or low relative humidity on infections caused by *Stemphylium vesicarium* on pear. The study indicated that Stemphylium infection occurred on pear plant at high humidity coupled with continuous wetness period. Whereas no infection was observed on plants incubated under high relative humidity without wetness.

Studies on artificial inoculation of *Allium* plants with *S. vesicarium* indicated that temperature of 18–26°C and a minimum leaf wetness period of 6 or 8 h is required for infection. However, increasing the wetness period to 24-48 h resulted in more consistent reactions, with larger number and size of lesions Prados *et al*. (2003).

Rossi *et al*. (2005) have studied the increase in spore concentration of *S. vesicarium* in pear which has been correlated significantly with the reduction of relative humidity and wetness in early morning, and the increase of wind in late morning and afternoon. Conidia of *S. vesicarium* became easily air-borne to form a regular component of the air-spora in pear orchards, while ascospores were found to be sporadic. They have emphasized on the significant correlation between spore peaks and days with favorable weather conditions defined as days with air temperature between 15 and 25°C and high humidity, particularly a wet period longer than 10 h. They have observed Stemphylium blight on garlic when the temperature ranged from 9-23°C and relative humidity 64-95%, respectively.

While conducting the epidemiological studies Mwakutuya (2010) observed temperature ranging between 25-30°C along with leaf wetness periods of 48 h favored the rapid Stemphylium blight progression in lentil.

The epidemiological studies conducted by Bhupatbhai (2015) on leaf blight in garlic variety GG-4 K revealed the significant severity in relation to meteorological parameters of high morning humidity and wind speed. Whereas, other variables like maximum temperature (X_1), minimum temperature (X_2) and evening humidity (X_4) did not have significant impact on development of leaf blight in garlic.

2.7 DISEASE MANAGEMENT

2.7.1 Varietal resistance

Pandey *et al.* (1989) while conducting varietal screening of garlic against Stemphylium blight and Purple blotch observed minimum disease per cent in variety HG-1 followed by G-1. However, varieties having dark green leaves were severely affected with Purple blotch and Stemphylium blight.

Bisht and Thomas (1992) conducted varietal screening trials on garlic during 1986-88 for assessing their resistance against purple blotch and Stemphylium blight under natural and artificial epiphytotic conditions. They have observed most of lines as moderately and highly susceptible to both the pathogens. Whereas, 9 lines namely IC-32320, -35286, -43398, -48157, -48875, -49415, EC-158250, T84/13 and C-1525 were found resistant to both the pathogens.

Six of the seven garlic cultivars/lines namely Blanco de Vallelado and Morado de Pedroneras exhibited varied susceptible response to inoculation with isolates 17/94 and 9/93 of *S. vesicarium.*

Suheri and Price (2000b) evaluated mean disease severity on 26 garlic accessions including the commercial cultivars. Of the garlic cultivars, California Early, Rojo de Castro, Rojo de Las Infantas, Thuma and Creole were ranked resistant. Australian White, Bhsto de Chinchon and Lokalen were found to be moderately resistant. Italian White, Arguni White, Rocamble and Chet's were susceptible and China town was evaluated as highly susceptible.

Among the 16 cultivars of garlic, G-1, G-4 and G-305 were found to be most promising against Stemphylium blight reflecting considerably less disease incidence (Srivastava *et al*., 2005).

Mishra *et al.* (2009) while screening different garlic lines against Stemphylium blight under field conditions observed a variable response among 21 lines G-294, G-324, G-351, G-368, G-369, G-176, and G-189 were rated as resistant, G-299, G-192, G-4 and G-323 as moderately resistant, G-222, G-54, G-213, G-366, G-264 as susceptible while G-266 was found highly susceptible.

Srujani *et al.* 2013 evaluated resistance levels of twenty two onion varieties under field conditions during 2010-2012 at Kalyani, West Bengal against *Alternaria porri* and *Stemphylium vesicarium*, the causal agents of Purple blotch and Stemphylium blight diseases. The disease severity was recorded using 0-5 rating scale and percent disease index was calculated. None of the 22 varieties, screened against Purple blotch & Stemphylium blight disease, were free from the disease. However, a significant variation existed among the varieties under study. While three varieties viz. NRCRO-4(1168), Sel.157 and COLL-652 were susceptible, varieties VG-18 and VG-19 were found to be moderately resistant and remaining 17 exhibited moderately susceptible disease reaction against Stemphylium blight only. Whereas, only one variety VG-18 performed best among all tested cultivars and found to be resistant and moderately resistance against both the diseases i.e. purple blotch and stemphylium blight, respectively.

2.7.2 Chemicals

Singh and Milne (1974) used poisoned food technique to evaluate the efficacy of 15 fungicides against *Stemphylium vesicarium* causing chrysanthemum flower blight and observed mancozeb, captafol, thiram and chloroneb as most promising fungicides.

Collina *et al.* (2006) observed the sensitivity of *Stemphylium vesicarium* to fungicides under *in vitro* conditions and evaluated dicarboximide as most effective against the pathogen. Fenbuconazole, penconazole, ziram and strobilurins were proved to be the most potential fungicides against conidial germination while difenoconazole, flutriafol and propiconazole exhibited the best inhibitory activity on mycelial growth.

Zheng *et al*. (2009) have reported inhibition of mycelial growth of *Stemphylium solani* by nine fungicides assayed on potato sugar agar medium. Flusilazole 40 EC had the highest inhibitory effect followed by that of difenconazole/propiconazole 30 EC, carbendazim 50 WP. The *in vivo* evaluation of fungicide revealed highest disease suppression by difenoconazole 10 WG followed by flusilazole 40 EC and flusilazole/famoxadone 67 EC was 92.0%, 90.0% and 90.0% respectively while efficiency of flusilazole/famoxadone 67 EC, difenoconazole 10 WG and flusilazole 40 EC were 54.0%, 45.7% and 41.4%, respectively.

Kumar *et al.* (2011) have tested ten fungicides against *S. vesicarium.* Among them, carbendazim, benomyl, mancozeb, vitavax, topsin-M and captan have proved to be the most effective in inhibiting the growth of fungus *in vitro.* Field spray of mancozeb (0.25%) on garlic crop at 15 days interval gave lowest disease intensity 9.8 per cent with highest yield (36.3 kg/plot) over control.

Galvez *et al.* (2016) while evaluating the effectiveness of nine fungicides of different chemical groups against Stemphylium mycelial growth found boscalid+ pyraclostrobin (succinate dehydrogenase inhibitors + quinone outside inhibitors) iprodione (dicarboximide) and prochloraz (demethylation inhibitors) highly effective in reducing radial mycelial growth even at low concentrations less than 5ppm with EC_{50} values.

Mishra and Singh (2017) while conducting *in vitro* study and field evaluation of fungicides for the management of Stemphylium blight and effect of fungicide on yield and quality of onion revealed that fluopyram 20% + tebuconazole 20% SC gave complete mycelial inhibition of test fungus at 50 ppm concentration. However,

strobilurins were found to be more effective, significantly reducing the disease and giving more than 50% disease control over check under field conditions.

2.7.3 Evaluation of different fungicides under field conditions

Basallote *et al.* (1998) obtained significant control of Stemphylium blight of garlic with foliar sprays of tebuconazole and procymidone used alone or alternated with chlorothalonil.

While evaluating the efficacy of two foliar spray of hexaconazole @ 0.1 % for two crop season, Barnwal (2003) observed a significant control of Stemphylium blight and a bulb yield of 190q/ha, net return of Rs. 7795.00 and Rs. 13290.00 per hectare with cost benefit ratio of 1:4.1 and 1:7.0, respectively. The above treatment was closely followed by two foliar sprays with Tetramethyl thiuram disulphide @ 0.2% which recorded cost-benefit ratio of 1:3.7 and 1:6.9, respectively.

Chander *et al*. (2004) during investigation reported appreciable control of chilli fruit rot (*Colletotrichum capsici*) and stemphylium blight (*Stemphylium botryosum*) with Antracol 70 WP (propineb), Folicur 250 EW (tebuconazole), Kitazin 48 EC (iprobenfos), Indofil M-45 (mancozeb), Kavach 75 WP (chlorothalonil), Score 25 EC (difenconazole), Contaf 5 EC (hexaconazole) and Bavistin 50 WP (carbendazim).

Lawande *et al.* (2009) reported that in garlic leaf blight diseases caused by *Alternaria porri, Colletotrichum* sp., *Stemphylium* sp. and *Cercospora* sp. singly or combined could be controlled by four sprays of mancozeb @ 0.3%, starting from 45 days after transplanting. Among the newer fungicides, two sprays of hexaconazole @ 0.1% were found most cost-effective.

Kishore *et al*. (2011) reported higher efficacy of Score (difenconazole 25 EC) 0.015% in managing Stemphylium blight of garlic over all other fungicides. This treatment was followed by Folicur (tebuconazole 250 EC 0.05% + Indofil M-45 (mancozeb 75 WP) 0.25%. Further they were compared in different schedules for their efficacy and economics against the disease. The schedule comprising of Companion (carbendazim 12% + mancozeb 63%) - Score (difenconazole 25 EC) - Indofil M-45 (mancozeb 75 WP) was quite effective (per cent disease control, PDC = 91.6) and high economical (cost benefit ratio, CBR = 1:3.12). This schedule was closely followed by schedule comprising of Indofil M-45 (mancozeb 75 WP) + Score (difenconazole 25

EC), Companion (carbendazim 12% + mancozeb 63% WP) (PDC = 85.84) with cost benefit ratio of 1:3.09 and 1:3.03, respectively.

Gupta and Gupta (2013) conducted a field evaluation of fungicide study on incidence and intensity of Stemphylium blight disease of onion and revealed that all tested fungicides significantly reduced the incidence and intensity of Stemphylium blight over control. The lowest Stemphylium blight intensity (3.87%) was achieved with sprays of propiconazole @ 0.1% followed by mancozeb @ 0.25%.

Nainwal and Vishunavat (2016) have reported six foliar spray of mancozeb @ 0.3% at fortnight interval was found to be the best and significantly superior in reducing Stemphylium blight disease intensity and increasing the yield of onion seed crop.

Chapter- 3

MATERIALS AND METHODS

The present study entitled "**Epidemiology and Management of Stemphylium blight of Garlic"** was carried out in experimental farm and laboratories of Department of Plant Pathology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during the year 2017 and 2018. The methodologies adopted during the course of studies are elaborated here under:

3.1 Survey and surveillance

3.2 Isolation and identification

3.3 Pathogenicity

3.4 Epidemiological studies

3.5 Disease management

3.6 Statistical analysis

3.1 SURVEY AND SURVEILLANCE

During the course of present investigations, different garlic growing areas of Solan and Sirmaur districts of Himachal Pradesh were surveyed for recording the disease severity during 2017-2018 crop season from February to April. The infected leaves of garlic showing the characteristics symptoms of Stemphylium blight were collected at regular intervals. The diseased specimens were critically examined through compound microscope in the laboratory for the presence of the causal organism. The collected diseased samples were stored in refrigerator at 5ºC for isolation of the pathogen. The data on per cent disease severity was recorded using (0-5) scale given by Srujani *et al.* (2013) with slight modifications as 0 = absolutely free from disease whereas 1, 2, 3, 4 and 5 corresponds to (1-10%), (11-20%), (21-30%), (31-60%) and (>60%) leaf area infected, respectively.

The per cent disease severity was worked out according to McKinney (1923) as given below:

$$(\%)\text{Disease severity} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings X Maximum disease grade}} \times 100$$

The plants showing characteristics symptoms of disease on leaves were collected in paper bags and brought to the laboratory for the isolation of test fungus.

3.2 ISOLATION AND IDENTIFICATION

3.2.1 Isolation

Pieces of infected leaves showing typical symptoms were used for the isolation of the test fungus by following standard tissue isolation method. Small bits of 1 to 3 mm size were taken from the junction of diseased and healthy portions with the help of sterilized blades. These bits were surface sterilized with mercuric chloride (0.1%) for 10 to 20 seconds and washed thrice with sterilized distilled water. The bits were transferred on sterilized filter paper to remove excess moisture and subsequently transferred to potato dextrose agar (PDA) slants under aseptic conditions. The inoculated slants were incubated for 4-5 days at 25±1^{o}C. Predominant colonies were re-isolated and purified by hyphal tip method and maintained on potato dextrose agar medium. Slants were preserved in a refrigerator at 5°C and revived once in 30 days to maintain purity of test fungus.

3.2.2 Identification

The morphological characters of the fungus were studied on the host as well as in culture grown on PDA. 10-15 days old culture was used for studying morphological characters of the test pathogen. Observations including mycelium colour, septation, conidia shape, colour and size (length and width), length of conidiophore, number of transverse constrictions, number of transverse and longitudinal septation were recorded using Olympus microscope and Promagnus software. These observations were compared with those of the standard measurements given by Simmons (1969) to identify the test fungus.

3.3 PATHOGENICITY

3.3.1 Preparation of inoculum

For inoculation, conidial suspension was prepared from 20 days old culture of the test fungus. The spores were harvested by flooding the culture plate with 10 ml of sterilized distilled water and gently scraping the surface with sterilized spatula to form spore suspension. The resulting conidial suspension was then passed through a double

layer of cheese cloth and concentration of $6x10^4$ conidia/ml was adjusted by adding sterilized distilled water with the help of haemocytometer.

3.3.2 Method of Inoculation

3.3.2.1 Pot culture

For conducting the pathogenicity test, plastic pots (10 cm dia.) were filled with sterilized soil. In each pot, 4 cloves of garlic cv. Local Selection were sown and test was conducted on 40 days old plant at 3-4 leaf stage. Leaves were injured by rubbing carborandum powder with the help of brush and thereafter rinsing the surface with sterile water. The conidial suspension prepared from 20 days old culture with strength of $6x10^4$ conidia/ml containing 0.1% Tween-20 was spray inoculated using a hand atomiser while on control treatment sterile distilled water was sprayed. The potted plants were covered with perforated polyethylene bags, moistened with water sprays to maintain high humidity and kept under artificial controlled conditions. As the symptoms of the disease appeared, the fungus was re-isolated from these plants and the re-isolated fungus was brought to pure culture, which was later compared with the original isolate.

3.3.2.2 Detached leaf technique

In order to prove pathogenicity of the test fungus which was found to be associated with leaf blight disease of garlic, fourth true leaves were taken from the garlic plants for inoculation. Surface of each leaf was injured by rubbing carborandum powder and the leaves were inoculated with a drop of 10µl conidial suspension $6x10^4$ conidia/ml. While, in control sterile distilled water was applied. The leaves were placed in 14 cm Petri dishes lined on both sides with double layered moist filter paper and incubated in relative humidity cum temperature control cabinet adjusted at relative humidity of >90% and temperature of 25 ± 1^oC. Leaves were observed for the development of small, irregular white flecks symptoms at regular intervals after inoculation. The incubation period with respect to inoculated plant parts (leaves) were worked out as follows:

$$P = t_s - t_i$$

Where,

P = incubation period in hours

t_s = time of appearance of initial disease symptom

t_i = time of inoculation of leaves

3.4 EPIDEMIOLOGICAL STUDIES

3.4.1 Effect of different inoculum concentration on disease development

In order to find out the optimum inoculum concentration required for disease development. Leaves of 40 days old potted plants were inoculated with conidial suspensions of different concentrations viz., $2x10^4$, $4x10^4$, $6x10^4$, $8x10^4$ conidia/ml as per (3.3.2.1). Simultaneously control treatment was also maintained. The pot plants were incubated in relative humidity cum temperature control cabinet at 25±1°C temperature and relative humidity of more than 90 per cent. Twelve plants were taken per replication and the treatments were replicated thrice. The incubation periods for inoculated leaves at different inoculum concentrations were recorded and per cent disease severity was recorded as per (3.1). The disease progression on different inoculum concentrations was measured by calculating apparent rate of infection (r) on the basis of area covered by the lesion as per Van der Plank (1963) using logistic equation as given below :

$$r = \frac{2.303}{t_2-t_1} \log_{10} \frac{X_2\,(1-X_1)}{X_1\,(1-X_2)}$$

where,

r	=	apparent infection rate per unit per hour
t_2-t_1	=	time interval between first and last observation
X_1 and X_2	=	proportion of leaf area covered by lesion at t_1 and t_2 time intervals, respectively
$(1-X_1)$, $(1-X_2)$	=	proportion of healthy leaf area at t_1 and t_2 time intervals, respectively

3.4.2 Effect of different leaf wetness periods on disease development

In order to find out the optimum duration of leaf wetness required for disease initiation by test pathogen, 40 days old potted plants at 3-4 leaf stage were inoculated as per (3.3.2.1) and were incubated in relative humidity cum temperature control cabinet at 25±1°C. After 0, 6, 12, 24, 48 and 72 h of wetness duration, inoculated pot plants were removed from the cabinet were air dried and observed for the initiation of disease symptom. The incubation periods for inoculated plants at different durations of leaf

wetness were recorded and per cent disease severity was recorded by the scale mentioned under (3.1). The disease progression at different leaf wetness periods was measured by calculating apparent rate of infection (r) on the basis of area covered by lesion as per Van der Plank (1963) using logistic equation (3.4.1).

3.4.3 Effect of different temperature on disease development

In order to find out the optimum temperature regime for the development of disease, 40 days old potted plants were inoculated as per (3.3.2.1) and incubated at different temperatures 10, 15, 20, 25 and 30ºC in separate sets in relative humidity cum temperature control cabinet adjusted at relative humidity of more than 90 per cent. The incubation periods for inoculated leaves at different temperature regimes were recorded and per cent disease severity was recorded as per (3.1). The disease progression at different temperature regimes was measured by calculating apparent rate of infection (r) on the basis of area covered by lesion as per Van der Plank (1963) using logistic equation (3.4.1).

3.4.4 Influence of Meteorological factors on Stemphylium leaf blight disease development

To study the role of meteorological factors on disease development, the cloves of garlic cv. Local Selection were sown in replicated plots during the first week of October at experimental farm of Department of Plant Pathology, Nauni during 2017-18 season. The leaf blight severity was recorded at weekly interval commencing from fourth week of January and continued up to last week of April. Simultaneously, meteorological data on temperature, relative humidity and cumulative rainfall were also recorded for the intervening period. Simple and multiple correlations were worked out separately to establish the relative contribution of these factors on the spread of the disease.

3.5 DISEASE MANAGEMENT

3.5.1 Germplasm Screening (Under controlled conditions)

Twelve lines/genotypes of garlic namely Local Selection, SG-17, Haryana Local Selection, SG-26 Bron, Kandaghat Selection, SG-18, SG-6, SG-27, SG-19, SG-20, SG-7 and SG-30 were screened against Stemphylium leaf blight disease under artificial epiphytotic conditions as per (3.3.2.1). Disease severity was assessed after 6, 9 and 12

days of inoculation and disease reaction of different genotypes was ascertain on the basis of 0-5 scale as given below:

Grade	Description	Disease reaction
0.	Free from disease	Immune (I)
1.	1-10% area of leaves/plant part infected.	Resistant (R)
2.	11-20% area of leaves/plant part infected.	Moderately Resistant (MR)
3.	21-30% area of leaves/plant part infected.	Moderately Susceptible (MS)
4.	31-60% area of leaves/plant part infected.	Susceptible (S)
5.	>60% area of leaves/plant part infected.	Highly Susceptible (HS)

3.5.2 *In vitro* evaluation of some fungicides against *Stemphylium vesicarium*

The relative efficacy of some systemic, non-systemic and combi fungicides was evaluated *in vitro* at four different concentration levels of 100, 250, 500 and 750 ppm by using poisoned food technique (Nene and Thapliyal, 1979). The lots of 50ml double strength PDA medium were prepared in 150ml flask and the medium was sterilized at 15-20 psi pressure for 20 minutes. Simultaneously, concentrations of different fungicides were also prepared in sterilized distilled water so as to get desired concentration of fungicides after mixing the fungicide solutions in the double strength media.

The poisoned media of different concentration of fungicides of the respective treatment were poured in 90mm Petri plates. After settling down of the media for 3-4 hour, the culture discs (3 mm dia.) cut from the margin of 20 day old vigorously growing culture of the test pathogen were placed in the center of each prepared Petri plate. A control was also maintained in which only plain sterilized water was added to double strength medium. Each treatment was replicated thrice and the inoculated plates were incubated at 25 ±1°C in BOD incubator. The colony diameter of test pathogen was recorded till the control plates were full with mycelial growth of the test pathogen. The per cent inhibition of mycelial growth at different test concentrations in relation to control was calculated by using the formula (Vincent, 1947) given as below:

$$I = \frac{C - T}{C} \times 100$$

where,

I = (%) Mycelial growth inhibition

C = Mycelial growth (mm) in control

T = Mycelial growth (mm) in treatment

The fungicides used are as follows:

Trade Name	Common Name	Chemical Name
Blitox 50	Copper oxychloride 50% WP	Copper oxychloride.
Dithane M-45	Mancozeb 75% WP	Manganese ethylene bis-dithiocarbamate + Zinc.
Companion	Carbendazim 12% + Mancozeb 63% WP	Manganese ethylenebis (dithiocarbamate) (polymeric) complex with zinc salt + Methyl benzimidazol-2-ylcarbamate.
Score	Difenconazole 25% EC	Cis-trans-3-chloro-4-[4-methyl-2, (1H-1,2,4-triazol-1-ylmethyl)1-,3-dioxolan-2-yl] phenyl 4 -chlorophenyl ether.
Tilt	Propiconazole 25% EC	Cis-trans-1-[2-(2,4-dichlorophenyl) -4-propyl-1,3-dioxolan-2-ylmethyl] -1H-1,2,4-triazole.
Contaf	Hexaconazole 5% EC	(RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol.
Folicur	Tebuconazole 25.9% EC	(RS)- 1-(4-Chlorophenyl)- 4,4-dimethyl-3-(1H, 1,2,4-triazol-1-ylmethyl)pentan- 3-ol.
Amistar 23	Azoxystrobin 23%	Methyl (αE)-2-[[6-(2cyanophenoxy) -4-pyrimidinyl] oxy] - α -(methoxymethylene)benzeneacetate.
Cabrio Top	Metiram 55% + Pyraclostrobin 5% WG	Methyl N-(2-(1-(4-chlorophenyl) 1H-pyrazol-3yl) oxy) methyl) phenyl) (methoxy) carbamate.
Bordeaux Mixture	Bordeaux mixture 0.8% (4:4:50)	Mixture of hydrated lime and copper sulphate.

3.5.3 Evaluation of fungicides against Stemphylium blight under field conditions

For evaluating the efficacy of fungicides a field experiment was laid out in the experimental farm of Department of Plant Pathology UHF, Nauni during 2017-18 crop seasons. Field trial on garlic local cultivar Local Selection constituting six treatments of fungicides (three systemic, one non systemic and two combi-fungicide) with one check were laid out in a Randomized Block Design (RBD) having a plot area of 1.0 m x1.5 m. Each treatment was replicated thrice. Garlic cloves were sown in the plots on 30-09-17 with a spacing of 15 cm between the row and 10 cm between the plants. The fungicides were sprayed three times, at 10 days interval started with the initiation of disease symptoms. Details of treatments applied are mentioned in table as given below:

Sr. No.	Treatments	Concentration (%)
1.	Cabrio Top	0.2
2.	Blitox	0.3
3.	Folicur	0.1
4.	Companion	0.25
5.	Tilt	0.1
6.	Score	0.1
7.	Control	Nil

Following were the observations taken to work out the relative efficacy of different treatments:

1. Disease severity: The disease severity was recorded in twelve randomly selected plants per replication as per the scale given by Srujani *et al*. (2013). Disease severity was calculated using the formula given by Mc Kinney (1923).

2. Bulb yield/plot: Bulb yield was recorded after the harvest of crop. The increase in yield in treated plot was determined by subtracting the yield of control plot from that of the treated plot. Per cent increase in yield was calculated using the following fornula.

$$(\%)\ \text{Increase in yield} = \frac{T - C}{C} \text{ X } 100$$

Where**,**

T = yield (kg/plot) in treated plot.

C = yield (kg/plot) in control plot.

3.5.4 Calculation of Incremental Cost Benefit Ratio (ICBR)

The incremental cost benefit ratio was calculated by taking the total yield per plot and converted in per hectare. The ICBR ratio denotes the proportion of extra amount obtained due to incremental yield in the treatment plot to that of the extra amount incurred for reducing the disease in respective treatment plot. The ratio has been calculated as per the following formula:

$$\text{ICBR} = \frac{\text{Extra amount obtained by treatment}}{\text{Amount incurred for disease management}}$$

Extra amount obtained = the income obtained on incremental bulb yield in treatment plot in comparison to control treatment plot.

Extra amount incurred for disease management in respective treatment = labour cost + cost of fungicides + depreciation cost

Where,

Labour charge Rs. 350/day (5 men/ha/spray)

Cost of fungicides = Folicur Rs. 1604/litre, Companion Rs. 250/500gm, Tilt Rs.125/100ml, Score Rs. 245/50ml, Cabrio Top Rs. 1260/600gm and Blitox Rs. 252/500 gm.

3.6 STATISTICAL ANALYSIS

The data recorded were analyzed by using MS-Excel and OPSTAT (Sheoran *et al.,* 1998). The mean values of data were subjected to analysis of variance as described by Panse and Sukhatme (2000) for using Randomized Block Design (Factorial) and Completely Randomized Design (Factorial).

Chapter-4

RESULTS AND DISCUSSION

The results of present study pertaining to the epidemiology and management of Stemphylium blight of garlic is interpreted under the following headings:

4.1 Survey and surveillance

4.2 Isolation and identification

4.3 Pathogenicity

4.4 Epidemiological studies

4.5 Disease management

4.1 SURVEY AND SURVEILLANCE

To study the occurrence of Stemphylium blight of garlic, systematic survey and surveillance studies of garlic growing areas of Solan and Sirmaur districts (Plate 1) of Himachal Pradesh were conducted during 2017 and 2018 cropping season at different stages of crop growth. The severity of disease was recorded as per 0-5 rating scale given by Srujani *et al.* (2013).

The results of the present survey and surveillance studies (Table 4.1) indicated that the Stemphylium blight of garlic was prevalent in almost all growing localities of Solan and Sirmaur districts. Disease severity ranged between 14.6 to 42.4 per cent and 33.7 to 48 per cent in Solan and Sirmour districts, respectively.

The perusal of data further indicated that the disease appeared in severe form in Karganu (48%) followed by Buttyudie (45.3%), Nauradhar (42.6%) and Mariyog (40%) of Sirmaur district, while it was in moderate proportion in Deedag (37.3%), Dharja (34.6%) and Chhogtali (33.7%) localities of the district. The stemphylium blight severity was recorded in moderate form in different garlic growing localities of Solan district viz., Lavighat (30.6%), Sihardi Brahmna (29.3%), Sandrol (24%) and Delgi (22.6%) while localities of the disrict Sirmaur viz., Sakot (19.3%), Berti (18.6%) and Bharech (14.7%) were considerably less affected by the disease.

Table 4.1 Status and distribution of Stemphylium blight of garlic in different localities of Solan and Sirmaur districts of Himachal Pradesh during 2018

District	Localities	Disease Severity (%)
SOLAN	Lavighat	30.6
	Sakot	19.3
	Sandhrol	24.0
	Berti	18.6
	Ghatti	25.3
	Chhausha	36.0
	Bharech	14.6
	Delgi	22.6
	Basholu	42.4
	Nauni	30.2
	Sihardi Brahmna	29.3
	Gadasar	35.8
	MEAN	**27.4**
SIRMAUR	Karganu	48.0
	Dhamla	39.4
	Mariyog	40.0
	Dharja	34.6
	Buttyudie	45.3
	Deedag	37.3
	Rajgarh	47.0
	Nauradhar	42.6
	Thadie	38.6
	Chhogtali	33.7
	Hanolipul	39.4
	MEAN	**39.5**

In the present study Stemphylium blight of garlic caused by *Stemphylium vesicarium* has been observed in severe form in the main pockets of garlic growing areas of Himachal Pradesh inflicting heavy damage to the crop in terms of loss in bulb yield and weight. Sugha and Kumar (2005) recorded incidence of Stemphylium blight of garlic during February, March and April in mild to severe form with average disease severity ranging from 37.85% – 65.5% in garlic growing localities of Himachal Pradesh. Katoch and Kumar (2016) also revealed high disease severity in Solan and Sirmaur districts of Himachal Pradesh. Therefore, the results of the present study are in evidence with the earlier studies on status and distribution of the disease.

Plate 1. Survey studies for Stemphylium blight of garlic in different localities of Himachal Pradesh

4.2 ISOLATION AND IDENTIFICATION

4.2.1 Isolation

The isolation of the test fungus associated with Stemphylium blight of garlic was made from the samples of infected leaves collected during survey and surveillance studies and pure culture was obtained on the PDA using the standard technique. The study revealed that the fungus protruded out in most of the bits cultured on the Petri plates which further developed off white mycelium. The isolated fungus was purified further by hyphal tip method. Purity of isolated fungus was regularly maintained and monitored by sub-culturing and the culture was preserved at 4±1^{o}C in refrigerator.

4.2.2 Identification

Diseased leaf samples exhibiting the characteristic symptoms of Stemphylium blight of garlic were visualized under microscope by cutting small transverse sections of diseased tissues. The pure culture isolates obtained from the diseased specimens of leaves were identified as *Stemphylium vesicarium* (Wallr.) E. Simmons (Plate 2) on the basis of the macroscopic and microscopic characteristics of the test fungus.

The identification of the isolated fungal species was further reconfirmed as *Stemphylium vesicarium* (Wallr.) E. Simmons through the courtesy of Dr. P.N. Chowdhary from National Centre of Fungal Taxonomy, New Delhi vide ID. No.93238.

As per the present morphological studies (Table 4.2), morphological characters of mycelium, conidia and conidiophore of *Stemphylium vesicarium* are being described as follows:

4.2.2.1Mycelium

In the initial stages of growth of the fungus, the mycelium was off white with faint yellow pigmentation in the center of the medium. The mycelium was septate, branched, raised, thin, smooth, olivaceous, golden yellow to medium or dark brown in colour (Plate 2a). The fungus grew velvety on PDA and was buff in colour. The fungus was slow growing and produced a yellow-brown pigment that turned deep red with age.

4.2.2.3 Conidiophore

Conidiophores were observed as straight to variously curved, simple, cylindrical but enlarging apically to the site of conidium production. The colour varied from diluted yellow

brown to golden brown at swollen apex (Plate 2c). Conidiophore measured 32.4 to 92.4 µm in length and 3.5 to 4.8 µm in width and the apical swelling measured 7.2-40.8 x 5.3-7.1 µm.

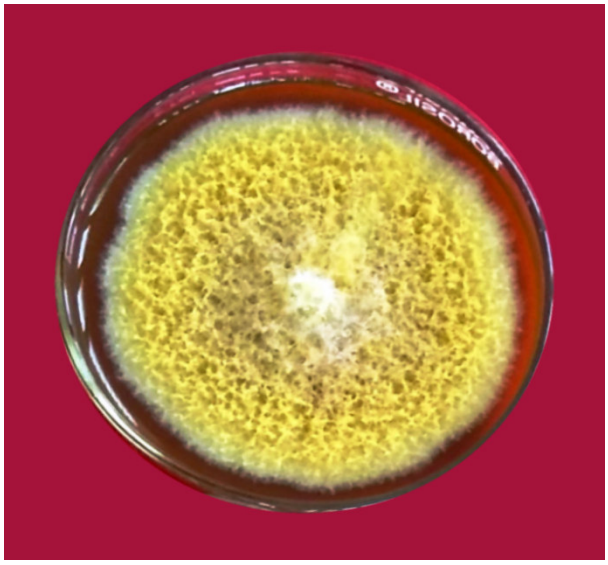
4.2.2.4 Conidia

Conidia were single, muriform, echinulate, medium golden brown to olive brown in colour and oblong or broadly oval to ellipsoidal in shape with 1-5 transverse and 1-2 complete series of longitudinal septa with 1-4 transverse constrictions (Plate 2b). The dimension of conidia was measured as 25-48x12-22 µm.

Table 4.2 Morphological characters of the test pathogen causing Stemphylium blight of garlic

Morphological characters		Description
Mycelium	**Type**	Septate, branched, thin, smooth and exhibited velvety texture
	Colour	Golden yellow to buff with faint yellow pigmentation in the centre
Conidiophore	**Type**	Straight, simple, smooth, curved, cylindrical and apex enlarging apically at site of production of conidia.
	Colour	Dilute yellow brown or golden brown that darkens to medium golden brown at the swollen apex
	Size (length x width)	32.4-92.4 µm x 3.5-4.8 µm
Conidia	**Shape**	Oblong to oval and broadly ellipsoidal, verrucose and slightly curved
	Colour	Medium golden brown to olive brown
	Size (µm)	25-48x12-22 µm
Number of transverse septa		1-5
Number of longitudinal septa		1-2
Number of transverse constrictions		1-3 upto 4

While conducting morphological studies on *Stemphylium vesicarium* (Wallr.) E. Simmons, the incitant of Stemphylium blight of garlic, various workers have also observed almost similar morphological characters of the pathogen (Simmons 1969; Aveling and Naude 1993; Boiteux *et al.,* 1994 and Basallote *et al.,* 1999).



Ventral Side

Dorsal Side

Plate 2a. Pure Culture of *Stemphylium vesicarium*

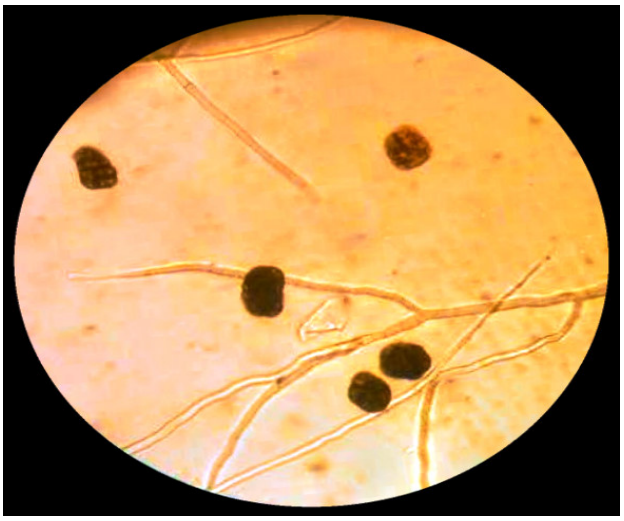


Plate 2b. Conidia

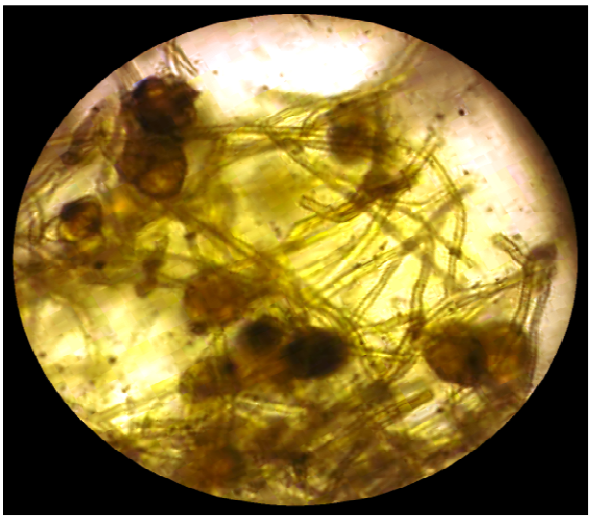


Plate 2c. Conidiophore

Plate 2. Morphological characters of *Stemphylium vesicarium* (Wallr.) E. Simmons

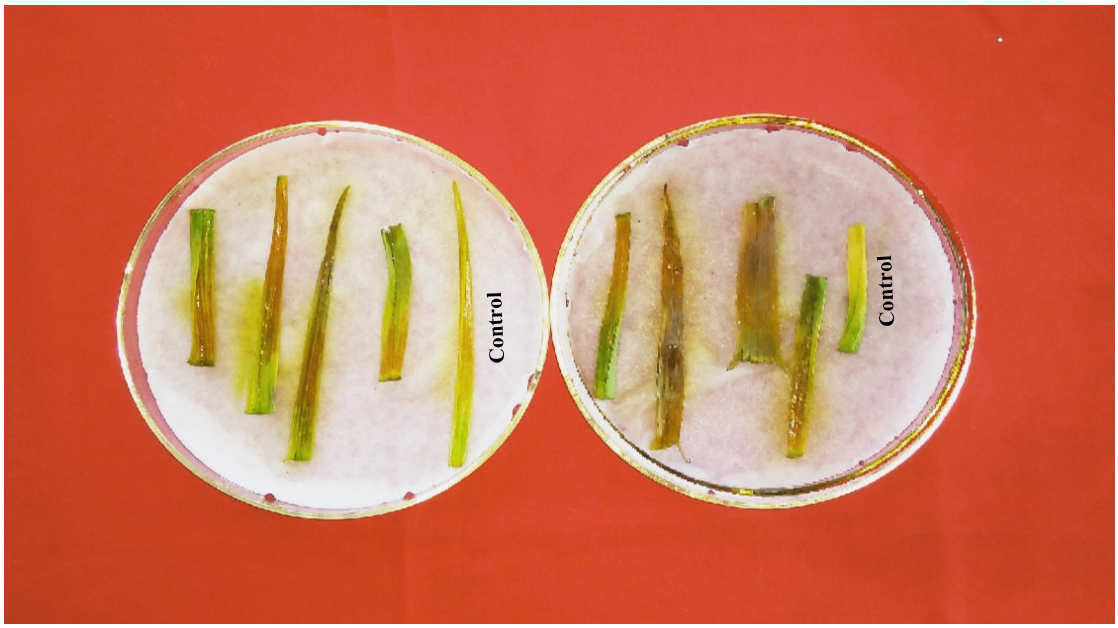


Plate 3a. Detached leaf

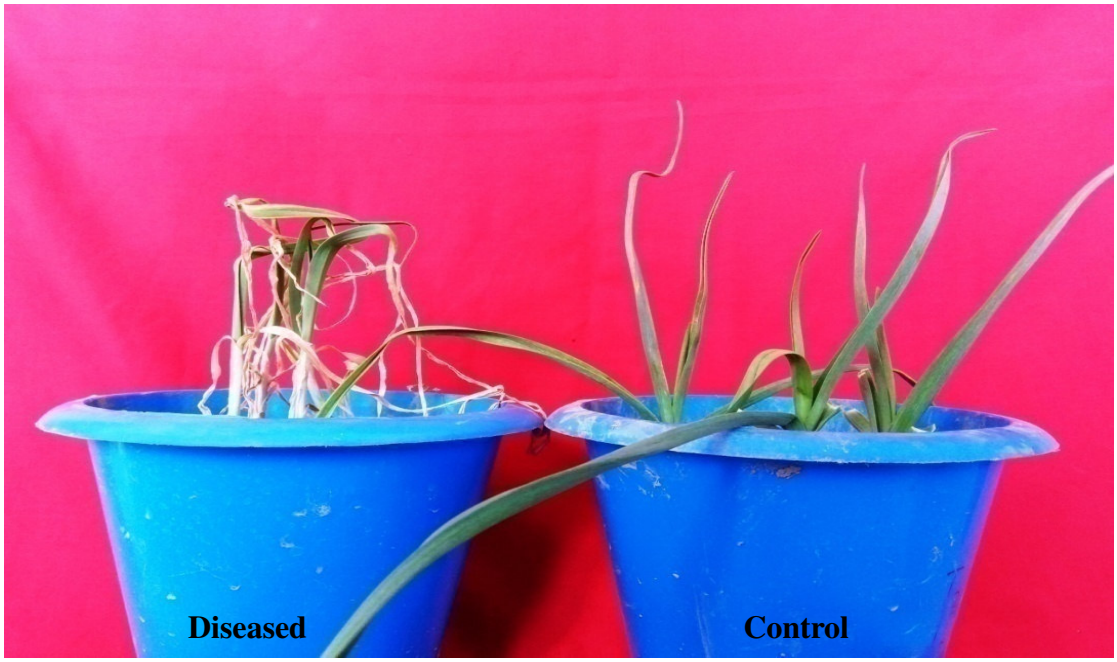


Plate 3b. Potted plants

Plate 3. Pathogenicity of *Stemphylium vesicarium* (Wallr.) E. Simmons on garlic plant/plant parts

4.3 PATHOGENICITY

4.3.1 Incubation period

Pathogenicity tests were conducted on garlic plants at 3-4 leaf stage by pot culture method and on fourth true leaf by detached leaf technique. During experiments, data on incubation period and symptom development was recorded and is presented in Table 4.3. The results indicated that initial symptoms appeared as small, irregular to oval, white flecks that were surrounded by a bright yellow margin. The incubation period was recorded as 96.2 h and 73.3 h after inoculation on detached leaf (Plate 3a) and potted plants (Plate 3b), respectively. However, no symptoms were observed on control plants.

While studying the pathogenicity of *Stemphylium vesicarium* on garlic under *in vivo* conditions, Basallote *et al*. (1999) also observed the initial disease symptoms after 4-6 days of inoculation in pot plants as minute, numerous white flecks on the leaves, whereas, control plants remained healthy throughout the period of experiment. Similarly, the pathogenicity of different isolates of *Stemphylium* sp., have been proved on various species of *Allium* by various workers (Shishkoff and Lorbeer, 1989; Suheri and Price, 2000b; Hassan *et al*., 2007 and Zheng *et al*., 2009).

Table 4.3 Pathogenicity of *Stemphylium vesicarium* (Wallr.) E. Simmons on garlic plant/ pant parts

Plant/plant parts		**Incubation period (h)**
Potted plants		96.2
Detached leaf		73.3
Symptom development	Initial stage	Appeared as small, numerous, irregular to oval, white to yellow flecks surrounded by bright yellow margin.
	Later stages	Flecks enlarged to produce zonated sunken lesion that turned light to tan brown in colour at centre measuring 1cm and expanded along leaf blade resulting in withered leaf tips.
	Advanced stages	Lesion turned dark olive brown to black as sporulation occured. Elongated spots coalesced frequently resulting in blightening of leaves.

4.3.2 Symptom development

Symptoms observed after the incubation period revealed that infected plants initially exhibited tip necrosis with white flecks that enlarged and produced sunken lesions, which were surrounded by a yellow to pale brown border.

In advanced stages these lesions measuring 0.5mm to 1cm in length usually turn light brown to tan at the centre and later dark olive brown to black as the pathogen sporulated. Finally, these lesions grew into elongated spots that frequently coalesced resulting in blightening of leaves (Plate 4d).

The fungus was consistently re-isolated from lesions developed within the inoculated leaves and was identified as *Stemphylium vesicarium* (Wallr.) E. Simmons on the basis of morphological characters and symptoms produced by test pathogen on the leaves, thereby proving the pathogenicity of the pathogen on garlic.

The symptoms observed during the present study were similar to that described by Zheng *et al*. (2009) as small, multiple irregular to oval white leaf spots which enlarged to produce sunken purple lesions surrounded by a bright yellow margin. Rao and Pavagi (1975) described symptoms on garlic and onion in later stage of infection as elongated spots that turned dark olive brown with the development of conidia and conidiophores of the pathogen. Hassan *et al*. (2012), Boiteux *et al*. (1994) and Aveling and Naude (1993) have also reported similar symptoms on garlic plants inoculated with spore suspension of *S. vesicarium*.

4.4 EPIDEMIOLOGICAL STUDIES

4.4.1 Effect of different inoculum concentrations on the development of Stemphylium blight of garlic

It is evident from data (Table 4.4) that there was considerable variation among different inoculum concentrations for incubation period. Per cent disease severity was recorded at 3, 6 and 9 days after recording the respective incubation periods of different inoculum concentrations. The inoculum concentration of $6x10^4$ conidia/ml was found optimum with incubation period of 85.3 h followed by $4x10^4$ conidia/ml (140.5 h) and $8x10^4$ conidia/ml (162.2 h). However, minimum infection on garlic leaves was found with $2x10^4$ conidia/ml suspension at 195.8 h of incubation period.

Plate 4a. Initial Stage **Plate 4b. Later Stage** **Plate 4c. Advanced Stage**

Plate 4. Stages of symptom development of Stemphylium blight of garlic in field.

Plate 4d. Characteristic blightening of garlic leaves caused by *S. vesicarium*

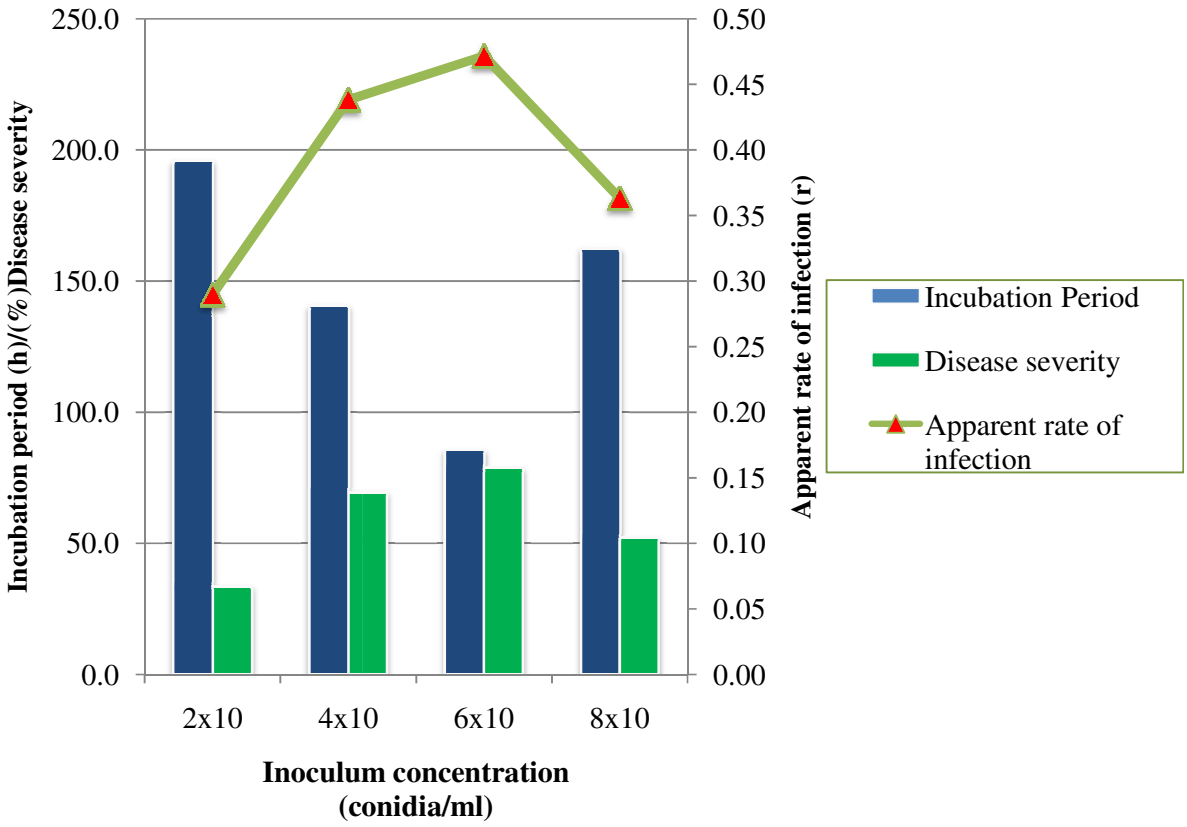


Figure 4.1 **Effect of different inoculum concentrations on the development of Stemphylium blight of garlic under *in vivo* conditions.**

The further persual of results (Table 4.4 and Fig. 4.1) on disease progression studies with respect to different inoculum concentrations revealed that $6x10^4$ conidia/ml concentration proved to be most favourable for disease development which was reflected by disease severity (78.60%) depicting highest apparent infection rate (0.47). This was followed by $4x10^4$ conidia/ml and $8x10^4$ conidia/ml resulting in 69.38% and 52.03% of disease severity. The apparent infection rate was recorded as 0.44 and 0.36, respectively. Considerably less disease severity (33.44%) was obtained with lowest conidial concentration of $2x10^4$ conidia/ml.

Table 4.4 Effect of different inoculum concentrations on the development of Stemphylium blight of garlic under *in vivo* conditions

Inoculum concentration (conidia ml^{-1})	Incubation period (h)	Disease severity (%) after days of incubation period			Mean	Apparent rate of infection (r)
		3	6	9		
$2x10^4$	195.8	8.13 (16.53)	15.94 (23.45)	33.44 (35.28)	19.17 (25.09)	0.29
$4x10^4$	140.5	14.07 (22.00)	32.66 (34.83)	69.38 (56.40)	38.70 (37.74)	0.44
$6x10^4$	85.3	17.82 (24.95)	38.28 (38.19)	78.60 (62.47)	44.90 (41.87)	0.47
$8x10^4$	162.2	10.94 (19.27)	24.38 (29.54)	52.03 (46.15)	29.12 (31.65)	0.36
Mean		12.74 (20.69)	27.81 (31.50)	58.36 (50.07)	-	

Figures in parenthesis are arc sine transformed values

$C.D._{0.05}$ Inoculum concentration (C) = 1.65 Time interval (I) = 1.43 CxI = 2.86

Results of present study on the effect of different inoculum concentration on disease initiation indicated $6x10^4$ as ideal inoculum concentration for rapid advancement of the disease. However, at $2x10^4$ conidial suspension infection process was noticeably delayed. Suheri and Price (2000a) while studying the effect of inoculum pressure of *S. vesicarium* on infection of leek leaves found out the noteworthiness of similar inoculum concentrations on development of disease symptoms on garlic and onion leaves.

4.4.2 Effect of leaf wetness periods on the development of Stemphylium blight of garlic

The results of the study (Table 4.5) revealed that incubation period for the disease development differed significantly at different leaf wetness periods. Leaf wetness duration of 72 h was found to be optimum with lowest incubation period of 73.3 h followed by 48 h with incubation period of 143.3 h, 24 h (165.2 h) and 12 h (190.8 h). Longest incubation period (212.5 h) was recorded at wetness period of 6 h. Whereas, upon inoculation without leaf wetness period the pathogen failed to develop any infection on leaves.

The perusal of data (Table 4.5 and Fig. 4.2) on disease development and apparent infection rate revealed that leaf wetness duration of at least 6 h was required for the development of Stemphylium blight disease and thereafter infection increased with increase in duration of leaf wetness. The pathogen resulted in higher disease severity (81.72%) at 72 h of leaf wetness followed by that of 48 h (71.72%), 24 h (53.44%), 12 h (32.66%) and 6 h (20.32%). Whereas, no infection occurred on the leaves incubated at 0 h of leaf wetness. The maximum and minimum apparent infection rate was recorded at 72 h (0.50) and 6 h (0.25), respectively.

Table 4.5 Effect of leaf wetness periods on the development Stemphylium blight of garlic under *in vivo* conditions

Leaf wetness (h)	Incubation period (h)	Disease severity (%) after days of incubation period			Mean	Apparent rate of infection (r)
		3	6	9		
0	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0
6	212.5	5.47 (13.48)	10.94 (19.27)	20.32 (26.75)	12.24 (19.83)	0.25
12	190.8	8.60 (17.02)	17.19 (24.45)	32.66 (34.83)	19.48 (25.43)	0.29
24	165.2	10.94 (19.28)	25.94 (30.57)	53.44 (46.96)	30.11 (32.27)	0.37
48	143.3	13.91 (21.88)	33.75 (35.49)	71.72 (57.87)	39.79 (38.41)	0.46
72	73.3	18.44 (25.40)	41.25 (39.94)	81.72 (64.72)	47.14 (43.36)	0.50
Mean		11.47 (19.41)	25.82 (29.94)	51.97 (46.23)	-	

Figures in parenthesis are arc sine transformed values

C.D. $_{0.05}$ Leaf wetness (W) = 1.46 Time interval (I) = 1.13 WxI = 2.53

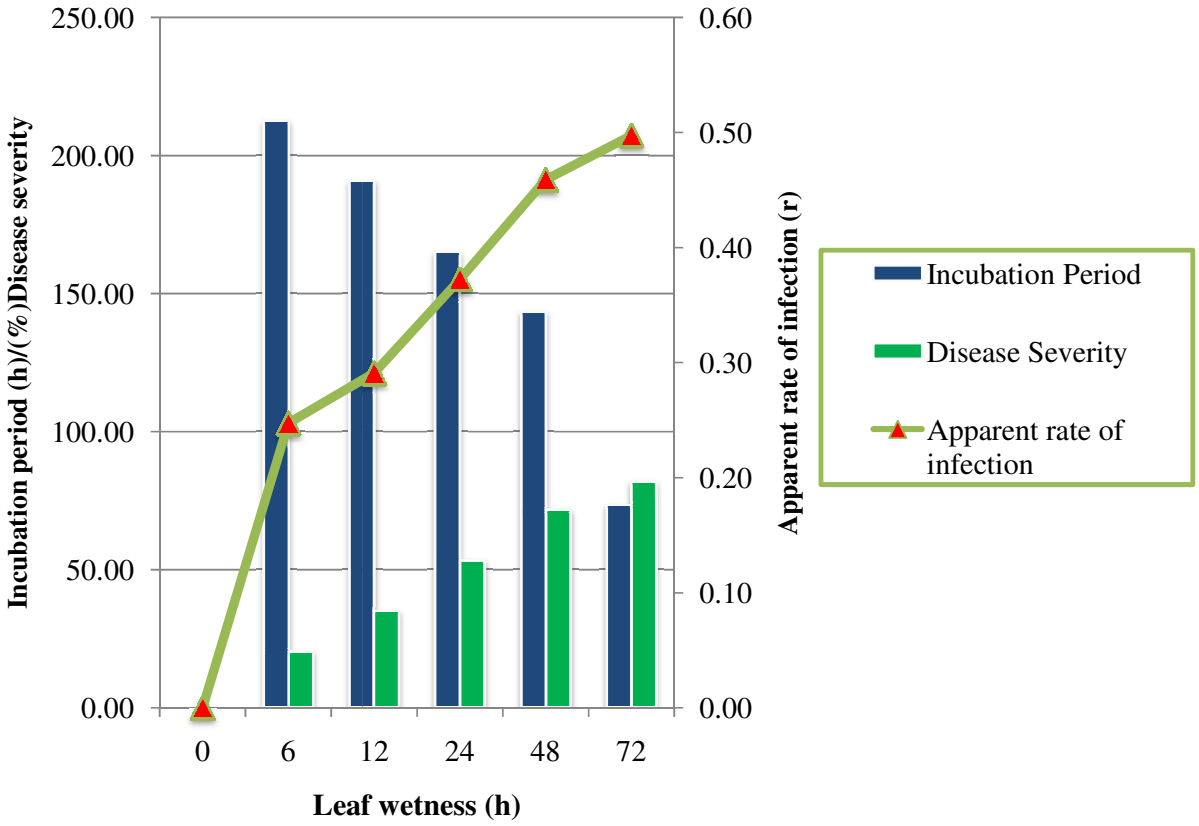


Figure 4.2 **Effect of different leaf wetness periods on the development of Stemphylium blight of garlic under *in vivo* conditions.**

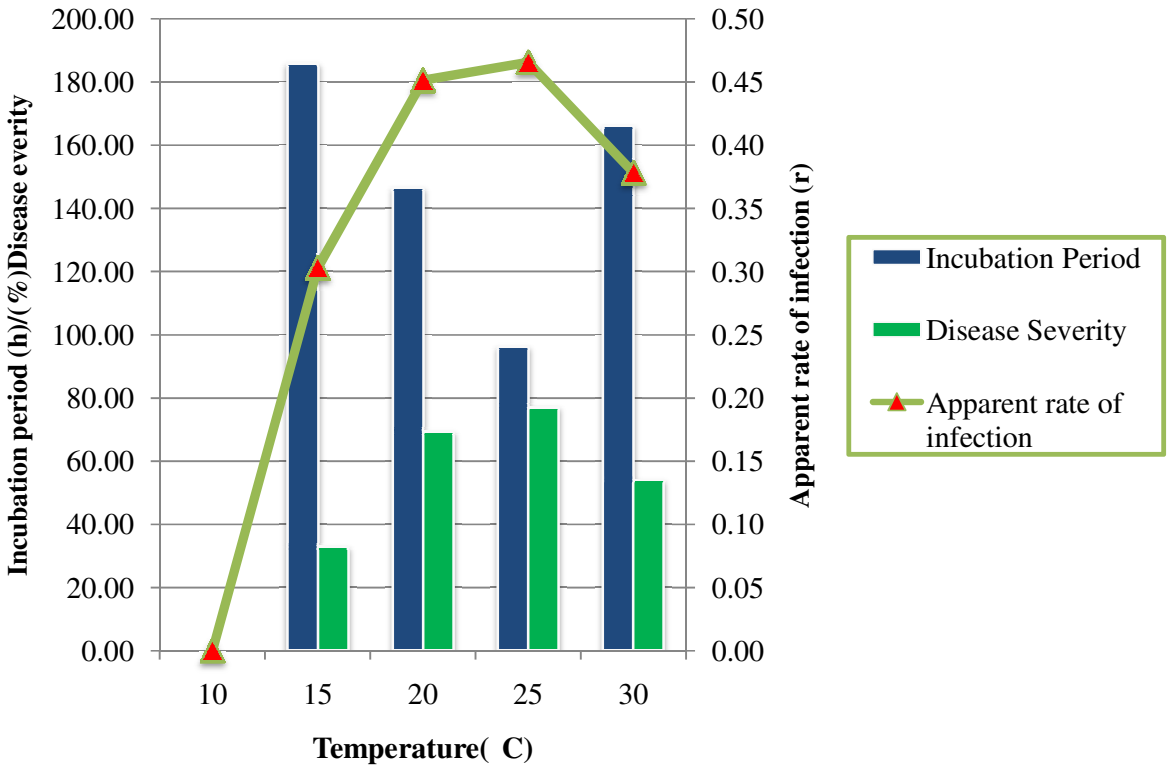


Figure 4.3 Effect of different temperature regimes on the development of Stemphylium blight of garlic under *in vivo* conditions

Therefore, the results of present study inferred that the disease appeared in increasing proportions with the increase in leaf wetness period and required at least 6h of leaf wetness for infection to take place. These results collaborated with the findings of Suheri and Price (2000b), Basallote-Ureba *et al.* (1999) and Prados *et al*. (2003) who also reported minimum 6 h of leaf wetness period required for initiation of Stemphylium blight in garlic plants.

4.4.3 Effect of different temperature regimes on the development of Stemphylium blight of garlic

The results of the study (Table 4.6) revealed that incubation period varied significantly at different temperature regimes being minimum at 25°C (96.2 h) followed by 20°C (146.5 h), 30°C (166.0 h) and 15°C (185.8 h). Although at 15°C the area covered by the disease was considerably less (32.82%) even after 9 days of incubation period. Whereas, at 10°C pathogen failed to develop any disease symptoms.

The further perusal of results (Table 4.6 and Fig. 4.3) indicated 25°C as optimum temperature for rapid disease progression reflecting maximum disease severity (77.03%) with high apparent infection rate (0.47). This was followed by that of 20°C reflecting disease severity (69.38%), 30°C (53.91%) and 15°C (32.82%). The minimum disease progression (32.82%) and apparent rate of infection (0.30) was recorded at 15°C.

Table 4.6 Effect of different temperature regimes on the development of Stemphylium blight of garlic under *in vivo* conditions

Temperature (°C)	Incubation period (h)	Disease severity (%) after days of incubation period			Mean	Apparent rate of infection (r)
		3	6	9		
10	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
15	185.8	7.35 (15.67)	17.19 (24.47)	32.82 (34.92)	19.12 (25.02)	0.30
20	146.5	13.13 (21.22)	33.28 (35.21)	69.38 (56.40)	38.60 (37.61)	0.45
25	96.2	17.03 (24.35)	39.38 (38.85)	77.03 (61.37)	44.48 (41.52)	0.47
30	166.0	10.78 (19.14)	20.94 (27.20)	53.91 (47.23)	28.54 (31.19)	0.38
Mean		9.66 (16.08)	22.16 (25.14)	46.63 (39.98)	-	

Figures in parenthesis are arc sine transformed values

C.D.$_{0.05}$ Temperature (T) = 1.28 Time interval (I) = 0.99 TxI = 2.22

Results of present studies are in collaboration with the observations recorded by Prados *et al.* (2003) who also reported temperature between 18°C to 25°C coupled with minimum leaf wetness period of 6 or 8 h as essential parameters for the development of the disease on garlic caused by *Stemphylium vesicarium*. Similar findings were also reported by various other workers for Stemphylium blight of garlic caused by *Stemphylium vesicarium* (Montesinos *et al*., 1995; Suheri and Price, 2000a; Rossi *et al.*, 2005 and Mwakutuya, 2010).

4.4.4 Effect of different meteorological factors on the progression of Stemphylium blight of garlic during 2017-2018 cropping season

The perusal of data (Table 4.7 and Fig. 4.4) on weather parameters and disease development revealed that the Stemphylium blight of garlic progressed more rapidly in the month of March, April and appeared prevailing during these two months in significant proportion during warm moist weather coupled with occasional rains in spring followed by dry and warm days. The disease progression was noticed at wide temperature and humidity range of 18.3°C-29.6°C and 40-60%, respectively. The disease progression was comparatively low during the month of January and February experiencing low temperature and dry weather conditions.

Table 4.7 Effect of different meteorological factors on the progression of Stemphylium blight of garlic during 2018 cropping season

Duration	Max Temp. (°C)	Min Temp. (°C)	Mean Air Temp. (°C)	Average Relative humidity (%)	Cumulative Rainfall (mm)	Leaf blight severity (%)
29-01-18 to 04-02-18	20.9	3.8	12.4	47	0.0	0
05-02-18 to 11-02-18	19.3	3.0	11.2	44	0.0	0.83
12-02-18 to 18-02-18	18.3	5.1	11.7	61	36.6	1.2
19-02-18 to 25-02-18	21.3	7.3	14.3	49	13.6	1.4
26-02-18 to 04-03-18	22.1	8.3	15.2	53	3.0	1.4
05-03-18 to 11-03-18	23.1	7.3	15.2	43	0.0	1.5
12-03-18 to 18-03-18	24.8	8.8	16.8	44	0.0	1.6
19-03-18 to 25-03-18	23.8	9.1	16.5	56	13.4	4.64
26-03-18 to 01-04-18	27.4	10.4	18.9	42	0.0	8.8
02-04-18 to 08-04-18	26.5	12.8	19.7	53	0.0	6.8
09-04-18 to 15-04-18	24.4	11.2	17.8	58.4	31.1	19.4
16-04-18 to 22-04-18	27.2	12.2	19.7	53.9	6.2	16.2
23-04-18 to 29-04-18	29.6	13.8	21.7	39.49	2.3	12.5

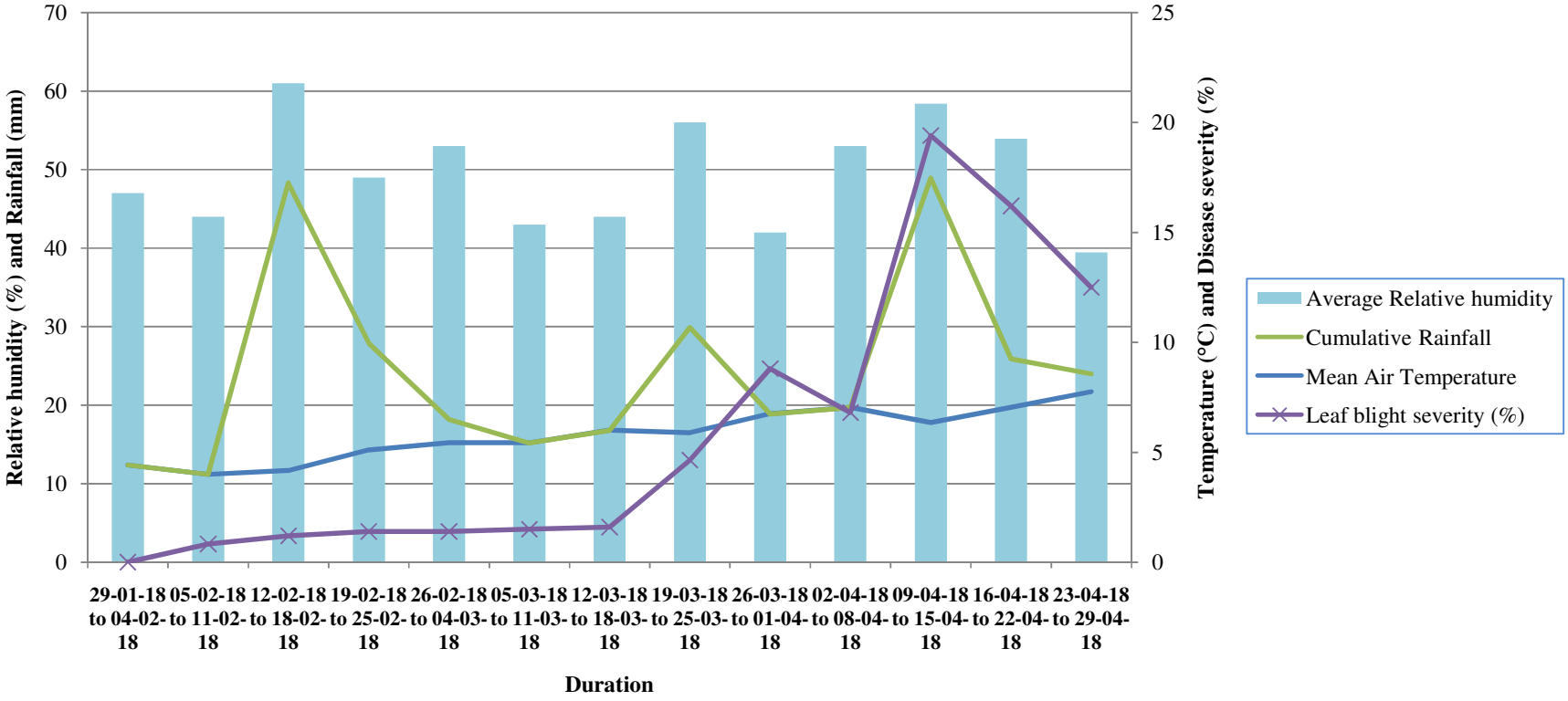


Figure 4.4 Effect of different meteorological factors on the progression of Stemphylium leaf blight of garlic during 2018 cropping season

4.4.4.1 Correlation of Stemphylium blight severity and incidence in relation to meteorological factors

Simple and multiple correlations were worked out on Stemphylium leaf blight severity with meteorological factors (mean air temperature, average relative humidity and cumulative rainfall) individually and collectively.

a) Simple correlations

Combined analysis of simple correlation coefficients worked out between Stemphylium blight of garlic and meteorological factors are presented in Table 4.8

Table 4.8 Simple correlation coefficients on Stemphylium leaf blight severity in relation to meteorological factors

Correlation pairs	Correlation coefficient
Disease severity x Temperature	0.7353
Disease severity x Relative humidity	0.1877
Disease severity x Cumulative rainfall	0.2470

The persual of simple correlation studies (Table 4.8) revealed that meteorological parameters (mean air temperature, average relative humidity and rainfall) on disease development of Stemphylium blight in garlic were positively correlated. The mean air temperature and cumulative rainfall has much more pronounced effect on the Stemphylium leaf blight development. The results of the present study inferred increase in Stemphylium blight development with the increase in temperature prevailing in March and April.

Bhupatbhai (2015) while conducting epidemiological studies of Stemphylium blight of garlic also indicated an increase in disease severity with increase in temperature. Similar findings have been reported by Kumar (2009) on Stemphylium blight of onion.

b) Multiple correlations

The coefficient of multiple correlation (R^2) was calculated to measure the contribution of linear function of independent variables, such as mean air temperature (X_1), average relative humidity (X_2), and cumulative rainfall (X_3) on dependent variable i.e. per cent disease severity (Y) presented in the table 4.9

The multiple correlation coefficient (Table 4.9) between disease severity and the independent variables was found to be 0.75 indicating the Stemphylium leaf blight progression in garlic was governed up to 75% by the cumulative effect of the meteorological parameters (temperature, rainfall and relative humidity) variation in Stemphylium leaf blight development.

Table 4.9 Multiple correlation coefficients on Stemphylium leaf blight severity in relation to meteorological factors and regression equation

R^2	Multiple coefficient of determination (%)	Regression equation
0.7500	75.00	$Y = 3.674 + 0.870\ X_1 + 3.294\ X_2 - 3.581\ X_3$
		Where, Y = Disease severity (%) X_1 = Mean air temperature (C) X_2 = Average relative humidity (%) X_3 = Cumulative rainfall (mm)

Significant at 5 per cent level of significance

The results of present studies were in confirmation with the findings of Gupta and Gupta (2014) also indicated the significant influence of weather parameters on Stemphylium leaf blight of onion.

4.5 DISEASE MANAGEMENT

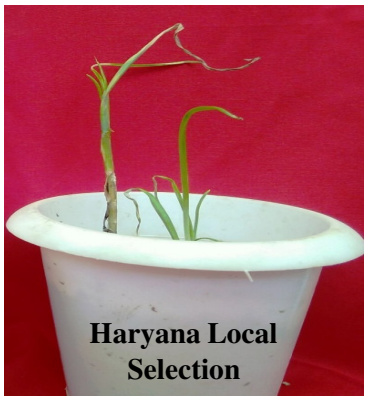
4.5.1 Germplasm Screening (Under controlled conditions)

Twelve genotypes were screened for their resistance reaction against Stemphylium leaf blight under artificial controlled conditions. The data on per cent disease severity and apparent rate of infection (r) corresponding disease reactions of different genotypes are given in Table 4.10.

From the perusal of data (Table 4.10) it is evident that none of the genotypes showed resistant reaction against Stemphylium blight of garlic. However, genotype Kandaghat Selection was rated as moderately resistant against the disease, reflecting lowest disease severity (15.56%) and apparent infection rate (0.19). The genotypes namely SG-17 and SG-18 were found to exhibit moderately susceptible reaction with disease severity of 25.93%, 22.59% and apparent infection rate of 0.26, 0.29, respectively. Haryana Local Selection, SG-26 Bron, SG-6, SG-27 and SG-20 were rated susceptible with severity level ranging from 50-60 per cent with apparent infection rate 0.32-0.39. Varieties Local Selection, SG-19, SG-7 and SG-30 reflected disease in significant proportion (>80%) and higher apparent infection rate (0.42-0.50) were rated as highly susceptible.

The results also revealed that none of the garlic genotypes/varieties tested was immune or resistant to the pathogen. Suheri and Price (2000b) reported genotypes namely

5a. Moderately Resistant **5b. Moderately Suseptible**



5c. Suseptible

5d. Highly Suseptible

Plate 5. Genotypes showing disease reaction against Stemphylium blight disease under artificial controlled conditions.

Australian White, Bhsto de Chinchon and Lokalen as moderately resistant against Stemphylium leaf blight disease of garlic. Bisht and Thomas (1992) observed most of the lines as moderately and highly susceptible to purple blotch and stemphylium blight under artificial and natural epiphytotic conditions.

Table 4.10 Reaction of garlic genotypes against Stemphylium leaf blight under artificial controlled conditions

Variety/ Genotype	Disease severity (%) after days of inoculation			Mean	Apparent Rate of infection (r)	Disease Reaction
	6	9	12			
Local Selection	22.96 (28.61)	44.08 (41.57)	85.56 (67.68)	50.86 (45.95)	0.50	**HS**
SG-17	6.67 (14.93)	13.33 (21.39)	25.93 (30.58)	15.31 (22.30)	0.26	**MS**
Haryana Local Selection	8.89 (17.32)	27.41 (31.54)	50.00 (44.98)	28.77 (31.28)	0.39	**S**
SG-26 Bron	13.70 (21.65)	33.70 (35.46)	59.26 (50.32)	35.56 (35.81)	0.37	**S**
SG-18	4.44 (12.10)	10.74 (19.09)	22.59 (28.35)	12.59 (19.85)	0.29	**MS**
SG-6	15.19 (22.89)	30.00 (33.20)	54.82 (47.75)	33.33 (34.61)	0.32	**S**
SG-27	17.41 (24.62)	34.44 (35.90)	60.00 (50.76)	37.28 (37.09)	0.33	**S**
Kandaghat Selection	0 (0.00)	5.55 (13.58)	15.56 (23.21)	7.04 (12.26)	0.19	**MR**
SG-19	29.26 (32.67)	55.18 (47.96)	84.44 (66.78)	56.30 (49.13)	0.43	**HS**
SG-7	25.92 (30.57)	52.96 (46.68)	81.48 (64.50)	53.46 (47.25)	0.42	**HS**
SG-20	12.22 (20.44)	24.82 (29.86)	53.33 (46.89)	30.12 (32.40)	0.35	**S**
SG-30	18.15 (25.18)	39.26 (38.78)	78.15 (62.14)	45.19 (42.03)	0.46	**HS**
Mean	14.57 (20.92)	30.90 (32.86)	55.77 (48.55)	-	-	-

Figures in parenthesis are arc sine transformed values

C.D. $_{0.05}$ Varieties/Genotypes (V) = 1.54 Time interval (I) = 0.77 VxI = 2.66

Where,

MR = Moderately Resistant MS = Moderately Susceptible

S = Suseptible HS = Highly Susceptible

4.5.2 *In vitro* evaluation

4.5.2.1 Efficacy of different fungicides under *in vitro* conditions against mycelial growth of *Stemphylium vesicarium* (Wallr.) E. Simmons causing Stemphylium blight of garlic

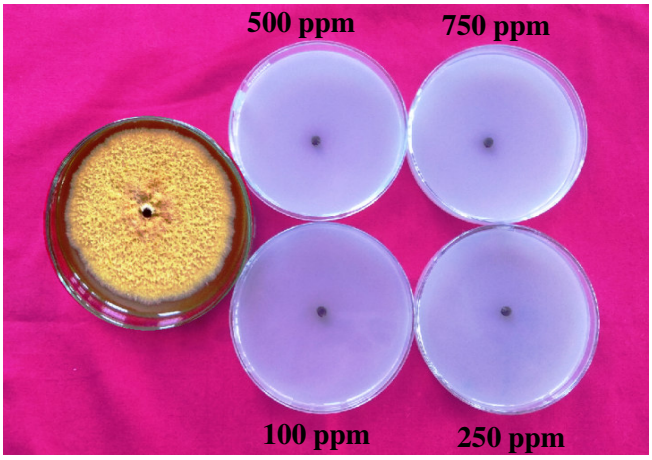
It is evident from the data (Table 4.11) that all the fungicides except Copper fungicides significantly inhibited the mycelial growth of the *Stemphylium vesicarium* (Wallr.) E. Simmons at recommended concentration as compared to the control. The data revealed that out of ten fungicides, Folicur and Companion completely inhibited (100%) the growth of *S. vesicarium* followed by Tilt and Cabrio-top results in 96.0% and 91.7% growth inhibition, respectively. The inhibitory response of Score (89.0%) and Contaf (88.7%) were found to be statistically at par. However, the inhibitory effect was comparatively less in Bordeaux mixture (21.3%), Blitox (21.7%) and Amistar (30.7%). Irrespective of different fungicides tested, the inhibitory response increased significantly with the increase in dosage levels from 100 to 750 ppm.

Table 4.11 ***In vitro*** **efficacy of different fungicides against mycelial growth of** ***Stemphylium vesicarium*** **(Wallr.) E. Simmons causing Stemphylium blight of garlic**

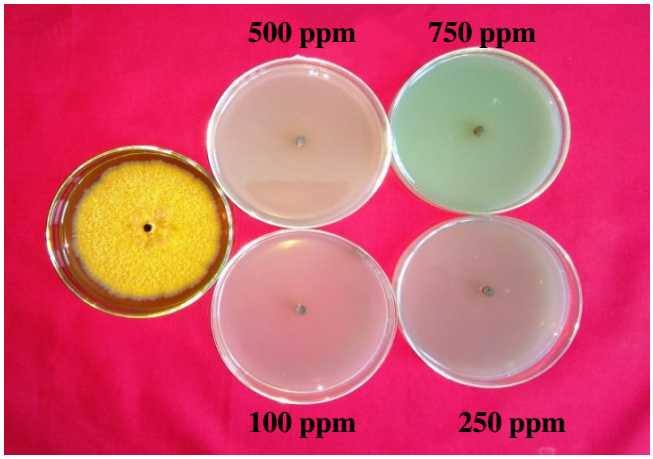
Fungicide	(%) inhibition at different concentrations				Mean
	100ppm	250ppm	500ppm	750ppm	
Cabrio Top	84.81 (67.04)	87.40 (69.19)	94.40 (78.27)	100.00 (87.98)	91.7 (75.62)
Score	87.40 (69.20)	87.59 (69.48)	88.51 (70.18)	92.55 (76.33)	89.0 (71.30)
Folicur	100.00 (87.98)	100.00 (87.98)	100.00 (87.98)	100.00 (87.98)	100.0 (87.98)
Mancozeb	65.55 (54.04)	75.92 (60.81)	87.03 (68.87)	100.00 (87.98)	82.1 (67.93)
Amistar	15.36 (23.05)	20.92 (27.20)	40.00 (39.20)	46.48 (42.96)	30.7 (33.10)
Blitox	10.37 (18.77)	13.33 (21.39)	24.44 (29.61)	38.51 (38.34)	21.7 (27.03)
Tilt	90.16 (71.73)	93.70 (75.43)	100.00 (87.98)	100.00 (87.98)	96.0 (80.78)
Contaf	81.48 (64.66)	82.77 (65.45)	93.51 (75.22)	96.96 (82.86)	88.7 (72.05)
Companion	100.00 (87.98)	100.00 (87.98)	100.00 (87.98)	100.00 (87.98)	100.0 (87.98)
Bordeaux mixture	5.55 (13.32)	14.81 (22.49)	25.18 (30.07)	39.81 (39.09)	21.3 (26.24)
Mean	64.07 (55.78)	67.64 (58.74)	75.31 (65.44)	81.43 (71.95)	-

Figures in parenthesis are arc sine transformed values

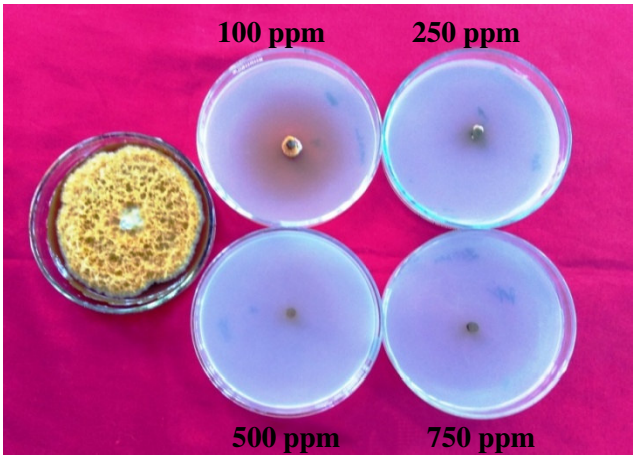
C.D.$_{0.05}$ Fungicides (F) = 2.49 Concentrations (C) = 1.57 FxC = 4.97



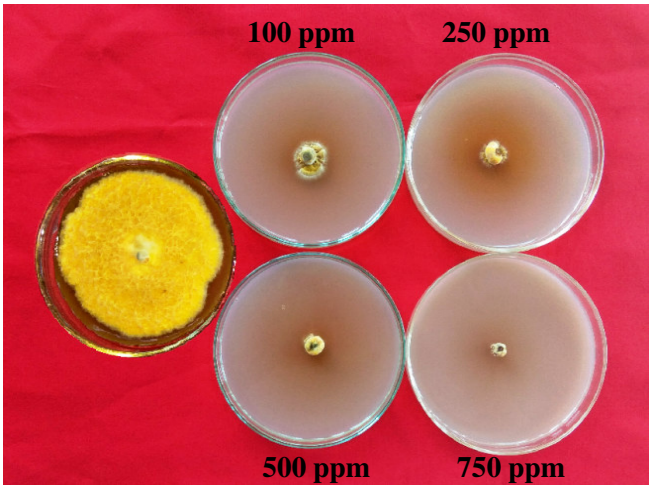
Folicur



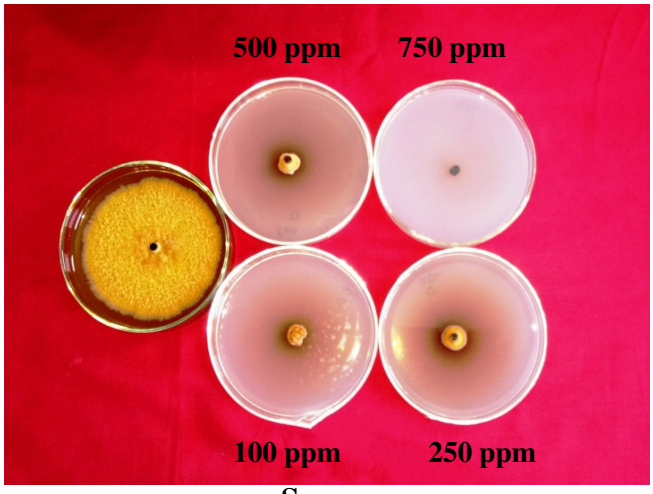
Companion



Tilt

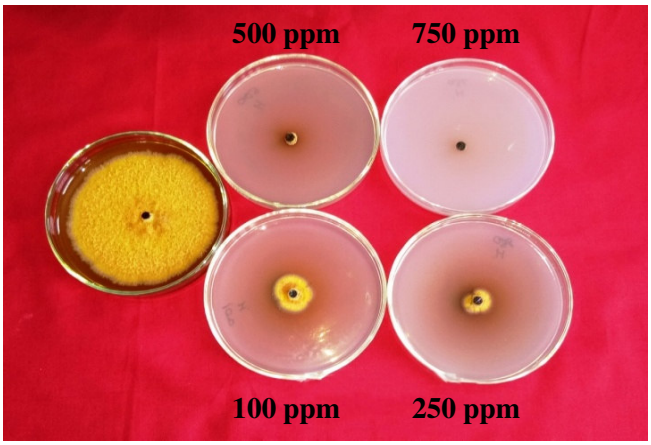


Cabrio Top

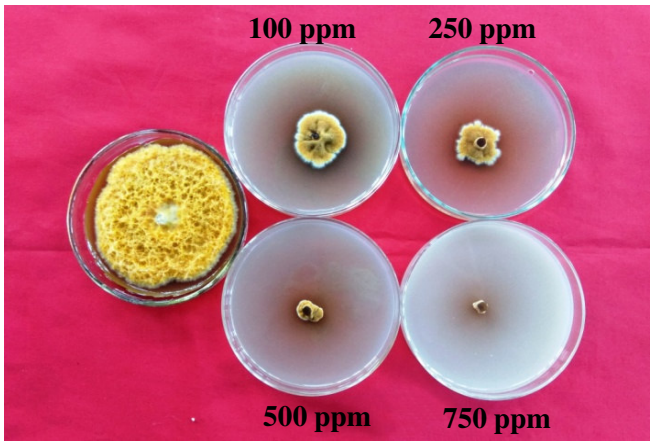


Score

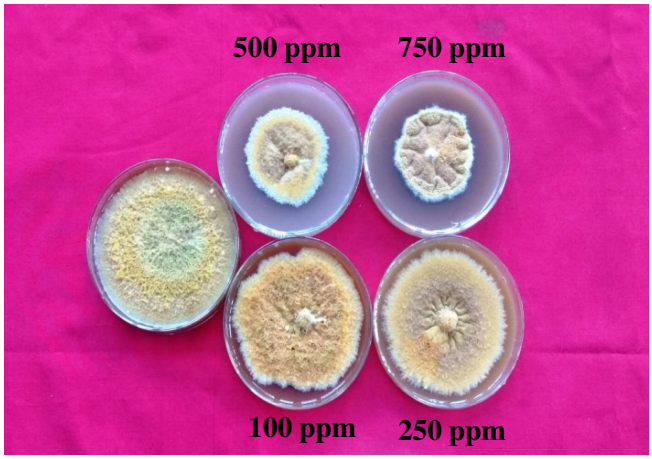
Plate 6a. ***In vitro*** **efficacy of systemic, non-systemic and combi-fungicides against** ***Stemphylium vesicarium*** **(Wallr.) E. Simmons**



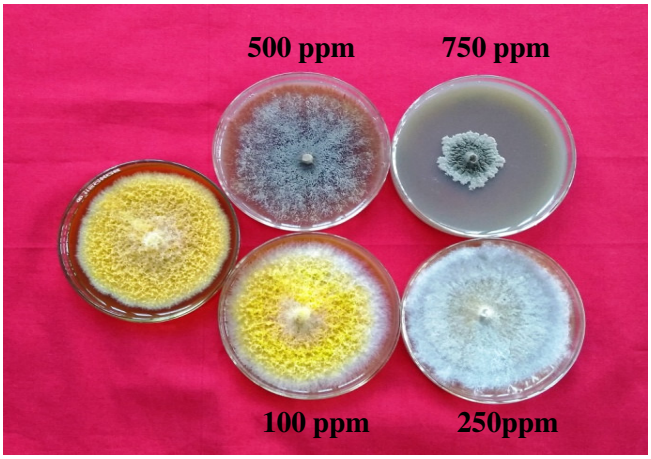
Contaf



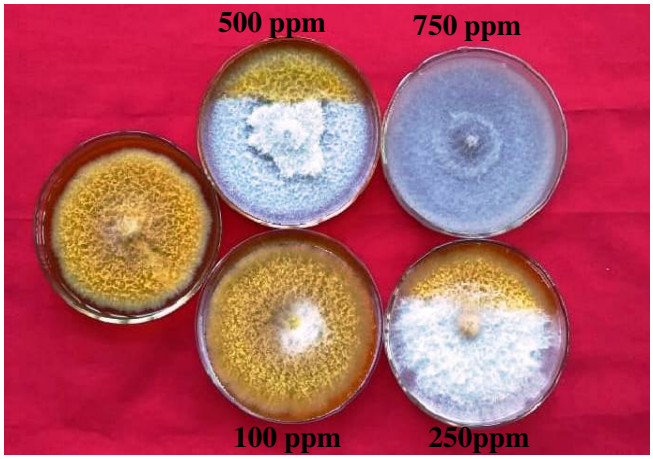
Mancozeb



Amistar



Blitox



Bordeaux mixture

Plate 6b. ***In vitro*** **efficacy of systemic, non-systemic and combi-fungicides against** ***Stemphylium vesicarium*** **(Wallr.) E. Simmons**

These findings are in conformity with the earlier study by Collina *et al*. (2006) who have also recorded tebuconazole, difenoconazole and propiconazole as most potent fungicides against mycelial growth of *S. vesicarium*. Chander *et al*. (2004) also reported the efficacy of triazole fungicides *in vitro* against *S. vesicarium* causing Stemphylium leaf blight of onion.

4.5.2.2 Field evaluation of different fungicides against Stemphylium blight of garlic caused by *Stemphylium vesicarium* (Wallr.) E. Simmons

Different fungicides (systemic, contact and combi-fungicides) were evaluated against Stemphylium blight under field conditions in Randomized Block Design in the experimental farm of Department of Plant Pathology, Nauni, during 2017-18 cropping season. Data on the per cent leaf disease severity, per cent disease control and bulb yield in each treatment plots were taken and the mean of which are being presented in the Table 4.12.

The results of study (Table 4.12) indicated that all tested fungicides were effective against Stemphylium blight of garlic to a variable extent under field conditions. The per cent leaf blight severity varied from 13.88–38.24 per cent in different treated plots as compared to 48.44 per cent in control. The study revealed that the application of three foliar sprays of Folicur @ 0.1 per cent at 10 days interval, started with the initiation of disease proved most efficacious (71.53%) in limiting Stemphylium blight disease and enhancing bulb yield (145.7q/ha) with minimum disease severity (13.88%) followed by Companion @ 0.25 per cent, Tilt @ 0.10 per cent and Score @ 0.10 per cent resulting in 68.93, 64.13 and 59.87 per cent reduction in disease severity, respectively. Blitox @ 0.30 per cent proved least effective (21.23%) in controlling the disease.

The present findings inferred the application of three foliar sprays either of Companion @ 0.25 per cent or Folicur @ 0.10 per cent at 10 days interval started with the initiation of disease as efficacious against Stemphylium blight disease. These results are in agreement with findings of Basallote *et al*. (1998) who have advocated good control of leaf spots in garlic caused by *Stemphylium vesicarium* with 4-9 foliar sprays of triazole fungicide (tebuconazole) during vegetative growth phase. Kishore *et al*. (2011) reported Companion as efficacious fungicide against Stemphylium blight of garlic while Gupta and Gupta (2013) recorded lowest Stemphylium blight intensity with four sprays of propiconazole @ 0.1%. Barnwal (2003) obtained significant control of Stemphylium blight with two foliar sprays of hexaconazole @ 0.1%.

Table 4.12 Efficacy of different fungicide against Stemphylium blight of garlic caused by *Stemphylium vesicarium* (Wallr.) E. Simmons under field conditions.

Treatment	Treatment detail	Conc. (%)	(%) Disease severity	(%) Disease control	Yield	
					(kg/plot)	(q/ha)
T_1	**Cabrio Top**	**0.20**	33.69 (35.37)	30.83 (33.30)	2.33	124.4
T_2	**Blitox**	**0.30**	38.24 (38.16)	21.23 (27.26)	2.23	119.0
T_3	**Folicur**	**0.10**	13.88 (21.72)	71.53 (57.86)	2.73	145.7
T_4	**Companion**	**0.25**	15.17 (22.73)	68.93 (56.25)	2.60	138.6
T_5	**Tilt**	**0.10**	17.51 (24.54)	64.13 (53.32)	2.53	135.0
T_6	**Score**	**0.10**	19.63 (26.02)	59.87 (50.80)	2.47	131.5
T_7	**Control**	**-**	48.44 (44.09)	-	2.13	113.7
	C.D. $_{0.05}$	**-**	**4.03**	**6.73**	**0.13**	**6.72**

Figures in parenthesis are angular transformed values

4.5.3 Incremental Cost Benefit Ratio

Incremental cost benefit ratio is the quick and easiest method to determine the margin by which an input is more beneficial or costly than another input. It is used to compare alternative options to help determine which is more feasible over the other. The incremental cost benefit ratio was calculated on the basis of cost of spray (including cost of chemicals/fungicides, labour cost per ha and depreciation cost) and increase in yield/ha over control. The data presented in Table 4.13 elucidated that among different treatments net income was highest (Rs. 1,27,920/ha) in T_3 (Folicur) followed by T_4 (Companion) (Rs. 99,493/ha) and T_5 in Tilt (Rs. 85,280/ha), whereas lowest net income (Rs. 21,320/ha) was calculated in T_2 (Blitox). However, all the chemicals were significantly better over control.

Maximum ICBR ratio (1:9.1) was obtained by T_3 (Folicur) followed by T_4 (Companion) (1:7.6) and T_5 (Tilt) with ICBR of 1:6.5 whereas minimum ICBR (1:1.5) was recorded in T_2 (Blitox). Higher net income in T_3 (3 sprays of Folicur @ 10 days interval) treatment contributed to the highest bulb yield of garlic. Similar findings were reported on garlic by Barnwal (2003) and Kishore *et al*. (2011). On onion by Gupta and Gupta (2013); Kumar (2009).

Plate 7a. A field trial laid out in RBD to determine efficacy of different fungicides to control Stemphylium blight disease

Tebuconazole (T_3)

Companion (T_4)

Control (T_7)

Plate 7b. Efficacy of different fungicides against Stemphylium blight of garlic under field conditions

Table 4.13 Incremental Cost Benefit Ratio of Stemphylium blight of garlic.

Fungicide/ Treatment	Yield Q/ha	Rate(Rs.) /Quantity	Fungicide Cost	Labour Cost	Depriciation cost	Total Cost	Gross Income	Net Income	ICBR
Cabrio Top T_1	124.4	1260/600gm	11340	5250	4500	21090	497467	42640	1:2.0
Blitox T_2	119.0	252/500gm	4082	5250	4500	13832	476147	21320	1:1.5
Folicur T_3	145.7	1604/1000ml	4331	5250	4500	14081	582747	127920	1:9.1
Companion T_4	138.6	250/500gm	3375	5250	4500	13125	554320	99493	1:7.6
Tilt T_5	135.0	125/100ml	3375	5250	4500	13125	540107	85280	1:6.5
Score T_6	131.5	245/50ml	13230	5250	4500	22980	525893	71067	1:3.1
Control T_7	113.7	-	-	-	-	**-**	454827	-	-

Chapter-5

SUMMARY AND CONCLUSIONS

The present investigation on **"Epidemiology and management of Stemphylium blight of garlic"** caused by *Stemphylium vesicarium* (Wallr.) E. Simmons was undertaken with regard to occurrence, identification, pathogenicity, epidemiology and management of Stemphylium leaf blight. The results obtained are summarized as under:

The Stemphylium leaf blight is an important disease of garlic in Sirmaur and Solan districts of Himachal Pradesh. During the course of disease survey of two districts viz. Solan and Sirmaur, disease was found to appear in moderate to severe form in garlic growing localities. The disease was recorded in severe form in localities viz., Karganu (48%), Nauradhar (42.6%) and Mariyog (40%) of Sirmaur district while it appeared in moderate proportions in Lavighat (30.6%) Sihardi Brahmna (29.3%) and Sandrol (24%) of Solan district.

The pure culture isolates obtained from the diseased specimens of leaves were identified as *Stemphylium vesicarium* (Wallr.) E. Simmons on the basis of the macroscopic and microscopic characteristics of the test fungus. The isolated fungus grew velvety on PDA and later appeared in the form of black pin head sclerotia that formed after 3 months at 25°C. Morphological studies revealed that the fungus was found to produce pigments at the beginning in the centre of the culture as faint yellow, which diffused beyond the margins of fungal growth and the colour darkened, ultimately to deep red. Mycelium of the fungus was septate, branched, raised, olivaceous and golden yellow to dark/medium brown in colour. Conidiophores were simple, straight to variously curved, cylindrical but enlarged at the swollen apex (the site of production of conidia). Conidia were single, muriform, verrucose, dilute/light brown or golden brown to olive brown in colour and oblong or broadly oval to ellipsoidal with 1-5 transverse and 1-2 complete series of longitudinal septa.

Pathogenicity of *S. vesicarium* was confirmed by detached leaf technique and pot culture method which revealed that initial symptoms appeared after 3 and 4 days of inoculation.

Characteristic symptoms of blight infected plants initially appeared in the form of tip necrosis with numerous small white flecks that enlarged and produced sunken lesions, surrounded by a bright yellow margin. Later, small light irregular to oval, yellow to brown, non-delineated lesions developed on leaves. These lesions turned light brown to tan in centre and later dark olive brown to black upon pathogen sporulation. Finally, these lesions elongated along the leaf blade, coalesced rapidly and resulted into complete blightening of the leaves.

Epidemiological studies revealed that incubation period varied significantly at different inoculum concentrations, leaf wetness periods and temperature regimes. The temperature of 25°C, leaf wetness period of 72 h and inoculum concentration of $6x10^4$ with >90% of relative humidity were found optimum for the disease development reflecting high infection rate.

The meteorological parameters in relation to weather variables indicated development of Stemphylium leaf blight of garlic was affected by variation in weather variables as well as by their interactions. The study indicated positive correlation of the disease progression with temperature, rainfall and humidity wherein these parameters contributed to the extent of 75.00% towards the development of Stemphylium blight.

In vitro evaluation of different systemic, non-systemic and combi fungicides revealed a variable inhibitory response of fungicides against the mycelial growth of *Stemphylium vesicarium*. Folicur (tebuconazole) and Companion (carbendazim+mancozeb) were found to be most efficacious resulting in cent per cent or complete inhibition of mycelial growth of the test pathogen followed by Tilt (propiconazole) (95.97%), Cabrio Top (metiram+pyraclostrobin) (91.65%), Score (difenconazole) (89.01%), Contaf (hexaconazole) (88.68%) and Dithane M-45 (mancozeb) (82.13%). The inhibitory response was comparatively less in Amistar (azoxystrobin) (30.69%), Blitox (copper oxychloride) (21.66%) and Bordeaux mixture (21.34%) as they were least effective in inhibiting mycelial growth of test pathogen at all concentrations. However, irrespective of different fungicides tested against mycelial growth there was a significant increase in inhibitory response with the increase in concentration.

None of the garlic germplasm screened was immune or resistant against Stemphylium blight under artificial controlled conditions. However, Stemphylium

blight was considerably less in variety Kandaghat Selection with lowest disease severity (15.56%) and rated as moderately resistant. The genotypes namely SG-18 and SG-17 were rated as moderately susceptible against the disease.

The fungicides evaluated under field conditions were efficacious to a variable extent in reducing the severity of Stemphylium blight. The treatment consisting of three foliar sprays of Folicur @ 0.1% at 10 days interval, started with the initiation of disease proved to be the most efficacious (71.53%) in limiting Stemphylium blight disease and enhancing bulb yield (145.7q/ha) with ICBR of 1:9.1 followed by Companion @ 0.25% with bulb yield (138.6q/ha) and ICBR 1:7.6 and Tilt @ 0.1% having 135.0q/ha bulb yield and ICBR 1:6.5 resulting in 68.93% and 64.13% reduction in disease severity, respectively.

The results in the present study can be concluded as under:

- Stemphylium blight of garlic was observed to be the major disease in Sirmaur district of Himachal Pradesh.
- The associated pathogen was isolated and identified as *Stemphylium vesicarium* (Wallr.) E. Simmons on the basis of cultural and morphological characters.
- The role of epidemiological conditions in disease development revealed that temperature of 25^{o}C, leaf wetness period of 72 h and inoculum concentration of $6x10^{4}$ with relative humidity of more than 90 per cent have been observed congenial for the progression of the disease.
- The meteorological parameters in relation to simple and multiple correlation studies revealed that mean air temperature 21.7ºC, average relative humidity (58.4%) and cumulative rainfall of 31.1mm resulted in maximum leaf blight severity (19.4%). All these parameters were found preferably most congenial for the progression of the disease.
- Out of 12 genotypes screened for disease reaction against Stemphylium blight disease of garlic under artificial epiphytotic conditions Kandghat Selection was found to be moderately resistant.
- *In vitro* evaluation studies revealed Folicur (tebuconazole) and Companion (carbendazim+mancozeb) as most efficacious fungicide resulting in complete inhibition of mycelial growth of *Stemphylium vesicarium.*

- Field evaluation studies revealed the application of three foliar sprays of Folicur @ 0.1 per cent as most efficacious in limiting the Stemphylium blight of garlic (71.53%) and enhancing the bulb yield (145.7q/ha) with ICBR ratio of 1:9.1 followed by Companion @ 0.25 per cent.

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APPENDIX-1

Culture media used for growing microorganism

1) Potato Dextrose Agar Medium

Peeled potato	:	250 g
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	1000 ml

APPENDIX II

ANOVA TABLES

ANOVA 1: Analysis of variance for effect of different inoculum concentrations on development of Stemphylium leaf blight on garlic under *in vivo* conditions. (Table 4.4 and Fig.4.1)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	2	7,069.067	3,534.534	895.969	0.00000
Factor B	3	1,930.620	643.540	163.131	0.00000
Intraction A X B	6	436.240	72.707	18.430	0.00000
Error	36	142.017	3.945		
Total	47	9,577.945			

ANOVA 2: Analysis of variance for effect of different leaf wetness periods on development of Stemphylium leaf blight on garlic under *in vivo* conditions. (Table 4.5 and Fig.4.2)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	2	7,300.116	3,650.058	1,160.302	0.00000
Factor B	4	4,334.830	1,083.707	344.495	0.00000
Intraction A X B	8	1,047.777	130.972	41.634	0.00000
Error	45	141.560	3.146		
Total	59	12,824.283			

ANOVA 3: Analysis of variance for effect of different temperature regimes on development of Stemphylium leaf blight on garlic under *in vivo* conditions. (Table 4.6 and Fig.4.3)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	2	5,826.992	2,913.496	1,203.713	0.00000
Factor B	4	12,886.708	3,221.677	1,331.039	0.00000
Intraction A X B	8	1,883.491	235.436	97.271	0.00000
Error	45	108.919	2.420		
Total	59	20,706.109			

ANOVA 4: Analysis of variance for effect of different meteorological factors on the progression of Stemphylium blight of garlic during 2018 crop season (Table 4.9 and Fig. 4.4)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated
Regression	3	1057.497	352.499	3.857
Residual	9	822.614	91.402	

ANOVA 5: Analysis of variance for reaction of garlic genotypes/varieties against Stemphylium leaf blight severity under artificial controlled conditions (Table 4.10)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	2	13,941.084	6,970.542	2,613.319	0.00000
Factor B	11	13,001.367	1,181.942	443.121	-0.00000
Intraction A X B	22	1,023.474	46.522	17.441	0.00000
Error	72	192.047	2.667		
Total	107	28,157.971			

ANOVA 6: Analysis of variance for *in vitro* efficacy of fungicides against mycelial growth of *Stemphylium vesicarium* (Wallr.) E. Simmons causing Stemphylium blight of garlic (Table 4.11)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Factor A	3	4,613.140	1,537.713	165.023	0.00000
Factor B	9	65,242.818	7,249.202	777.962	0.00000
Intraction A X B	27	2,167.716	80.286	8.616	0.00000
Error	80	745.456	9.318		
Total	119	72,769.129			

ANOVA 7: Analysis of variance for efficacy of fungicides on Stemphylium leaf blight severity on garlic under field condition (Table 4.12)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	2	196.905			
Treatment	6	1,379.674	229.946	98.901	0.00000
Error	12	27.900	2.325		
Total	20	1,604.478			

ANOVA 8: Analysis of variance for efficacy of fungicides on controlling Stemphylium leaf blight on garlic under field condition (Table 4.12)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	2	484.568			
Treatment	5	2,500.275	500.055	115.943	0.00000
Error	10	43.130	4.313		
Total	17	3,027.973			

ANOVA 9: Analysis of variance for efficacy of fungicides on yield (kg per plot) of garlic under field condition (Table 4.12)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	2	0.001			
Treatment	6	0.807	0.134	27.327	0.00000
Error	12	0.059	0.005		
Total	20	0.867			

ANOVA 10: Analysis of variance for efficacy of fungicides on yield (quintal per ha) of garlic under field condition (Table 4.12)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Signficance
Replication	2	2.801			
Treatment	6	2,291.747	381.958	27.339	0.00000
Error	12	167.655	13.971		
Total	20	2,462.202			

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ABSTRACT

Stemphylium blight caused by *Stemphylium vesicarium* (Wallr.) E. Simmons is an important destructive disease among various other diseases. It appears every year in moderate to severe form in different garlic growing areas of Solan and Sirmaur districts of Himachal Prdaesh. The causative fungus isolated from infected leaves of diseased plants grew in the form as off white mycelium, which later turned golden brown and finally velvety on PDA. Conidia were oblong to oval, light to olive brown in colour, produced on straight to variously curved cylindrical conidiophores. Thus, based upon morphological and cultural characters, the causative fungus was identified as *Stemphylium vesicarium* (Wallr.) E. Simmons. The pathogenicity tests revealed peculiar symptoms as small white to yellow flecks which later turned into lesions tan brown in colour and expanded along the leaf blade, giving blighted appearance to the leaves. Epidemiological studies revealed that the temperature of 25ºC, inoculum concentration of $6x10^4$ conidia/ml, leaf wetness period of 72 h and relative humidity of more than 90 per cent were optimum for the rapid progression of disease reflecting significant higher infection rate under artificial epiphytotic conditions. Under field conditions, disease was affected by variation in weather variables, as dry and moist weather coupled with intermittent rains favoured the progression of Stemphylium leaf blight disease. Twelve genotypes were tested against Stemphylium blight disease of garlic. None of the genotypes tested was found immune or resistant to the disease whereas, Kandaghat Selection was found moderately resistant against the disease. *In vitro* evaluation of fungicides indicated Folicur (tebuconazole), and Companion (carbendazim+mancozeb) as potent mycelial growth inhibitors of *S. vesicarium.* Field evaluation studies inferred the application of three foliar sprays of Folicur (tebuconazole) @ 0.1 per cent at ten days interval started with the initiation of disease to be most efficacious in limiting Stemphylium blight of garlic (71.5%) and enhancing the bulb yield (145.7q/ha) with ICBR ratio of 1:9.1.

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