“STUDIES ON LEAF SPOT OF CARNATION”

A thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,

RAHURI-413 722, DIST. - AHMEDNAGAR

MAHARASHTRA, INDIA

By

MISS. PALLAVI DHANAJI GHADAGE

Reg. No. 015/254

In partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND

AGRICULTURAL MICROBIOLOGY ,

COLLEGE OF AGRICULTURE, PUNE-411005

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Approved By

Dr. P. D. Mahajan
(Chairman and Research Guide)

Dr. T. K. Narute	**Dr. B. B. Chirame**	**Dr. D. S. Kakade**
(Committee member)	(Committee member)	(Committee member)

DEPARTMENT OFPLANT PATHOLOGY AND

AGRICULTURAL MICROBIOLOGY SECTION

COLLEGE OF AGRICULTURE, PUNE – 411005

MAHARASHTRA (INDIA)

2017

CANDIDATE'S DECLARATION

I hereby declare that this thesis entitled **"STUDIES ON LEAF SPOT OF CARNATION"** or part thereof has not been previously submitted by me or any other person to any other University or Institute for a Degree or Diploma.

Place: Pune

Date : / /2017 **(PALLAVI D. GHADAGE)**

Dr. P. D. Mahajan
Assistant Professor of Plant Pathology,
College of Agriculture, Pune - 411005
Maharashtra State (INDIA)

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON LEAF SPOT OF CARNATION"** submitted to the Mahatma Phule Krishi Vidyapeeth, Rahuri for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE**) in **PLANT PATHOLOGY**, embodies the results of a *bonafide* research work carried out by **MISS. PALLAVI DHANAJI GHADAGE** under my guidance and supervision. No part of the thesis has been submitted for any other Degree or Diploma.

Place : Pune **(Dr. P. D. Maahajan)**

Date : / /2017 Chairman and Research Guide

Dr. P. N. Rasal
Associate Dean and Principal
College of Agriculture, Pune-411005
Maharashtra State (INDIA)

CERTIFICATE

This is to certify that the thesis entitled, **"STUDIES ON LEAF SPOT OF CARNATION"** submitted to the Mahatma Phule Krishi Vidyapeeth, Rahuri for the award of the degree of **MASTER OF SCIENCE** (AGRICULTURE) **in PLANT PATHOLOGY,** embodies the results of a *bonafide* research work carried out by **MISS. PALLAVI DHANAJI GHADAGE** under the guidance and supervision of **Dr. P. D. MAHAJAN**, Professor of Plant Pathology, College of Agriculture, Pune – 411005 and that no part of the thesis has been submitted for any other Degree or Diploma.

Place : Pune

Date : / / 2017 **(Dr. P. N. Rasal)**

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"…Gratitude is the due respect right from the heart…"

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Place : Pune

Date : / / 2017 **Pallavi Dhanaji Ghadage**

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LIST OF ABBREVIATIONS

%	:	Per cent
µm	:	Micrometer
i. e.	:	Illustrated example (That is)
°C	:	Degree Celsius
C. D.	:	critical difference
cm	:	Centimeter
DIA	:	Day after inoculation
DAT	:	Day after transplanting
dsm^{-1}	:	Deci simon per meter
EC	:	Electric conductivity
et al	:	Et all (any other)
etc.	:	Et cetera (and others)
Fig	:	Figure
g	:	Gram
ha	:	Hectare
Lit.	:	Literature
Min.	:	Minute
ml	:	Milliliter
mm	:	Millimeter
MPKV	:	Mahatma Phule krishi Vidyapeeth

MT	:	Million tonnes
No.	:	Number
PDA	:	Potato dextrose agar
PDC	:	Per cent disease control
PDI	:	Per cent disease incidence
pH	:	Puissance de hydrogen
RH	:	Relative humidity
S. E.	:	Standard error
Spp	:	Species
Var	:	Variety
Viz	:	Videlicet (namely)

ABSTRACT

"STUDIES ON LEAF SPOT OFCARNATION"

By

MISS. PALLAVI DHANAJI GHADAGE

A candidate for the degree

Of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI - 413 722

2017

Research Guide	**:**	**Dr. P. D. Mahajan**
Department	**:**	**Plant Pathology and Agricultural Microbiology**
		College of Agriculture, Pune - 411 005

Carnation (*Dianthus caryophyllus* L.) is one of the most important commercial flowers of the world and is valued for its cut flowers. Amongst the diseases of carnation, *Alternaria* leaf spot is most damaging one, causing great losses. In view of the commercial importance of carnation flowers and their medicinal values, the present investigation was undertaken with regards to isolation of the pathogen and its pathogenicity, symptomatology, *in vitro* evaluation of bio-agents and botanicals and integration of effective bio-agents and botanicals to manage *Alternaria* leaf spot of carnation, in pot culture.

The pathogenicity of isolated fungus was tested by leaf inoculation method. Typical symptoms of the *Alternaria* leaf spot disease observed after week, concentric rings were formed as a result of irregular growth pattern by the organism in the leaf tissue. On the basis of pathogenicity and morphological characters, the test organism was identified as *Alternaria dianthi.*

The bio-agents evaluated *in vitro* revealed maximum mycelial growth inhibition with *Trichoderma viride* and *T. hamatum*, respectively of 73.01 and 72.15 per cent. Among the botanicals tested in *in-vitro*, aqueous extracts of Clove @ 10 per cent recorded highest mycelial growth inhibition (91.58%), followed by Neem leaf @ 10 per cent (56.07%), Garlic clove @ 10 per cent (49.53%) the *Alternaria dianthi.*

In vivo pot culture evaluation revealed the treatment of spray with of Clove extracts @ 10 per cent as most effective with highest leaf spot disease control (90.01%), followed by Neem leaf extracts @ 10 per cent (65.90%), Garlic clove extracts @ 10 per cent (62.73%), *T. viride* @ 0.5 per cent (52.53%) and *T. hamatum* @ 0.5 per cent (44.99%).

The varieties Eskimo and Gwen were found moderately resistant to *Alternaria* leaf spot.

Thus, it is concluded that foliar spray with Clove extract @ 10 per cent could effectively control *Alternaria* leaf spot of carnation.

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1. INTRODUCTION

Carnation (*Dianthus caryophyllus L.*) is one of the important cut flower crops of the world, rank second with commercial importance, next only to rose and cut carnation contribute about 50 per cent of the world cut flower trade (Jawaharlal *et al.,* 2009). Carnation is preferred for export owing to its excellent keeping quality, wide range of forms, colours and ability to withstand long distance transportation. Carnations are excellent for cut flowers, bedding, pots, borders, edging and rock gard fens. In western countries such as USA, it ranks next only to roses in popularity. Carnation is the national flower of Spain. Carnation is cultivated widely on a large scale in Italy, Spain, Columbia, Kenya, Sri Lanka, India, Canary Islands, France, Holland, Germany and USA (Haouala and Zid 2005). India with varied climatic zones offers a tremendous scope for its cultivation where farmers cultivate carnations for cut flower market (Casas *et al.,* 2010). Total area and production of cut flower in India during 2013-2014 were about 210ha. and 6000 MT, respectively (Anonymous, 2014).

Carnation (*Dianthus caryophyllus* L.) is indigenous to the Mediterranean region. It belongs to the family Caryophyllaceae that has 88 genera and 1750 species. Carnations have been cultivated for over 2000 years ago. Modern varieties of carnations were developed first in France in 1840 (Ali *et al.,* 2008). The name "Carnation" probably must have come from the Greek word "Coronation" or "Corone" (flower garlands), as it was one of the flowers used in Greek ceremonial crowns. The genous name Dianthus is derived from Greek “dios”, “divine” “anthos”, flower which means 'Flower of Zeus' or Divine flower. Fifteen is the basic chromosome number of Dianthus. Carnations are annuals or evergreen perennials, flowering prolifically in midsummer. The plants are medium tall about 45-90 cm. high, with long, narrow and thick grass like smooth leaves. The stems

are grey-green, hardy, shiny, swollen nodes and have one to three angles with tumid joints. Inflorescence is generally a terminal cyme, sometimes racemiform. Flowers are bisexual and occasionally unisexual. The flower that borne on the terminal ends of the stems may be single or double with fringed or smooth- edged petals and various colours like white, purple, red, pink, yellow, rosy, crimson, buff, lavender, cherry, apricot, salmon, maroon.Flower colour in carnations is produced by two different pigment types: carotenoids and flavonoids. The carotenoids, are responsible for colours ranging from yellow to orange. Anthocyanins are water-soluble pigments derived from flavonoids (Zuker *et al.,* 2002). Wild type carnations have the anthocyanin spelargonidin (orange, pink, red) and/or cyanidin (red, magenta), but do not naturally synthesise delphinidin (blue or purple). Flower fragrance in carnations is predominantly due to eugenol, beta-caryophyllene and benzoic acid derivatives. Allergy to carnation may be associated with IgE-mediated reactions in rhinitis and occupational asthma. Causal relationships between carnation allergen exposure and asthma symptoms in carnation workers have been observed (Sanchez-Guerrero *et al.,* 1999).

Ideal soil for cultivation of carnation is organic matter rich sandy laom soils with pH of 5.5 - 6.5 and EC of 0.8 - 1.2 dSm-1 during the vegetative stage and 1.2 - 1.5 dSm-1 during the generative stage.Commercial cultivation of carnation is followed by both seeds and vegetative methods. Because of high demand in floriculture market and susceptibility of many cultivars to fungal and viral phytopathogens, there is a pressing need for healthy and true-to-type planting material of carnation cultivars (Kayamori *et al.,* 2012). Multiplication of commercial carnation cultivars through adventitious shoot regeneration methods, facilitates availability of good, disease-free planting material.Temperature is the major factor that influences

the growth and flowering of carnation. Best quality carnations are produced in areas having high light intensity during winter and at the same time the temperatures during summer months are mild. Ideal day and night temperature is 28 ºC and 16 to 18ºC, respectively. The carnation is a facultative long day plant, which means that they form the flowers faster during long days than in short days. At the initial stages of growth and development, humidity should be maintained around 80 to 85 per cent. Whereas at full growth stage it should be 60 to 65 per cent. Optimum CO_2 concentration should be around 800 to 1000 ppm. Pinching and disbudding are an important cultural operation in the successful production of top quality carnations.

Although cut carnations are sold in western countries all the year round, they are in particular demand for the Valentine's Day, Easter, Mother Day and Christmas. While standard carnations are great demand, the miniature types are fast gaining popularity for their potential role in floral arrangement and also as cut flower at comparatively low price. White and pink standard carnations are in the greatest demand followed by red, yellow and bicolour. The liking for colour depends upon the time or season. In certain parts of France and Holland the carnation flowers are also used for the extraction of perfume. Only light coloured flowers are useful for this purpose. The volatile oil contains 30 per cent eugenol, 7 per cent phenyl ethyl alcohol, 40 per cent benzyl benzoate, 5 per cent benzyl salicylate and 1 per cent methyl salicylate Carnation absolute is used in sophisticated perfumes. Besides aesthetic value, carnation flowers are considered to be cardiotonic, diaphoretic and alexiteric (Thakur *et al.*, 2002; Kumar *et al.*, 2006).

Diseases are one of the most limiting problems that affect production of cut flowers. The number of diseases has increased due to increase in

acreage and also as a result of continuous importation of propagative material often infected from different parts of the world. At the same time, some changes in the relative importance of some diseases have been observed, which is probably due to the management given to these diseases and the introduction of susceptible varieties. In the present communication attempt has been made to cover the various diseases that affect carnation world over and for convenience these diseases have been described under three heads *viz.*, vascular pathogens, stem and root rots, and foliar diseases. Carnation is affected by number of disease like *Fusarium* wilt, rust, bacteria wilt, foot rot, leaf spot, powdery mildew, carnation mottle virus and carnation ring spot dianthovirus which causes huge losses in yield and quality of flower. Alternaria leaf spot is the common foliage disease of carnation and is caused by *Alternaria dianthi.* The fungus causes spots on the leaves and stem so that the leaves blight and die premature. *Alternaria* leaf spot/blight (*Alternaria dianthi* Stevens & Hall) it is a major disease affecting carnations. The initial symptoms are small purple lesions produced on leaves which later turn to greyish-brown. On expansion the lesions merge and result in blighting of leaves. The infections are generally noticed on lower leaves just above the ground level and gradually progress upwards. Greyish-brown lesions are also seen on stems. The disease appears mainly during rooting time. On plants grown for flower production, the symptoms appear in the first few months after transplanting. The optimum temperature and pH for *A. dianthi* are 25-30°C and pH 6 for vegetative growth while for spore germination it ranged from 20-25°C. Under natural conditions symptoms appear in December-January and are most severe during July-October. Disease severity is significantly correlated with the minimum temperature. The level of disease decreases as planting date is delayed. Therefore the study was undertaken with following objective;

Objectives :-

1. To prove Koch`s postulate.

2. To evaluate bio-agents & botanicals against leaf spot pathogen *in-vitro.*

3. To evaluate effective bio-agents & botanicals against leaf spot *in-vivo.*

4. To screen available genotypes of carnation against leaf spot

2. REVIEW OF LITERATURE

2.1 The Pathogen

Steven and Hall (1909) reported that *Altrenaria dianthi* reproduces asexually, forming row like spores of hyphe. Spores are formed on blisters on the host as well as within the mycelium. The thin, globular spores are spread through water. *Altrenaria dianthi* was first reported in scientific literature by them.

Rodriguez and Juan (1980) stated that *Altrnaria dianthi* fungus has been cultured on simple media such as potato dextrose agar and does not need dianthus tissue to germinate. Study found that spores would not germinate below 55 per cent humidity. The life cycle takes about four days from germination to the production of new conidia. Spot of this disease is smaller than one centimeter, but can be larger especially around stems.

Albajes (1999) described spores produced by *Altrenaria dianthi* species are black and can persist on dead tissue, in soil and on hard surfaces such as those found in green house.

Chun Fang Duan *et al.*, (2015) reported of *Alternaria dianthicola* causing flower blight on Carnation in China. Initial symptoms of carnation blight on flowers and immature flower buds were characterized by water-soaked areas. Spots with purple margins could be seen on petals of some varieties. An *Alternaria* spp. was isolated from 100 per cent of symptomatic tissues sampled. The isolates showed high virulence when inoculated on to tissue culture plantlets and on healthy carnation flowers. Results of sequence analysis of 5.8S rDNA-ITS of isolates showed 99 per cent sequence similarity with *Alternaria dianthicola* in gen bank.

Edwards (2017) evaluated *Alternaria alternata* causing leaf spot on golden aster a greenhouse-grown plant exhibited symptoms of disease

that included elongated brown lesions on leaves and leaf dieback. Club-shaped conidia (n = 20) that contained both longitudinal and transverse septa were produced in linear and branched chains and had an average length of 17.09 μm (11.86 to 23.91 μm) and an average width of 9.88 μm (6.27 to 12.6 μm).

2.2 Isolation, Symptomatology and pathogenicity

Watkins (1981) observed *Alternaria* leaf spot small, dull to dark brown, circular or irregularly shaped spots appear on leaf varying in diameter from 0.5 to 10 mm. They often develop concentric rings and represent a target board like appearance which is better defined on the upper surface. When mature they have dry, grey centres which may crack and even drop. The spots may coalesce and occupy large area of the leaf.

Seung-Hun *et al.*, (1989) reported that *Alternaria dianthi* infection begin as small circular or ovular spots on leave and stem, which can be red, purple , brown, yellow or gray. The multicolour circular spots can grow to infect entire plants resulting in wilting or death. The fungus has been cultured on simple media such as potato dextrose agar and does not need tissue to germinate.

Sharma (1994) studied *Alternaria dianthi* in carnation and other Dianthus culture in India. Brief description of symptoms and methods of control are presented for the following disease of carnation in India *viz. Alternaria dianthi, Fusarium oxysporum* f.sp. *dianthi, F. roseum, F. tricinctum,* etc

Tomioka *et al.,* (2000) tested the lesion morphology on both African marigold and French marigold was quiet similar. The conidiophores were mononematous without branches, straight to flexuous with 1-8

transverse septa, somewhat swollen at the bases, scarred at the apices, brown, smooth, 36-144µm in length , 6-10µm in width and 8-10µm in width at the basal swellings. Conidia were monotretic at the apics of the conidiophores, 4 enteroblastic and hydrophobic, dry airborne spores, rarely two in chains, brown multiseptate, obclavate with a single break at the tops. Conidial beaks were straight or slightly curved without branches, rarely scarred at the apices, 2-7 septa, 80-164µm in length, 5-8µm in width at the bases and tapering to the apices.

Gupta (2002) observed the symptoms of leaf blight and flower blight of marigold on African marigold under natural conditions. Initially the disease appeared as minute brown to deep brown, circular to oblong, necrotic spots on the older leaflets towards their margin and tips. The adjacent spots later coalesced with one another to form large irregular patches. Concentric rings were also seen in some old bigger old spots.

Chand *et al.,* (2007) described the symptomatology and morphological characters of *Alternaria* spp. (*A. brassicae, A.brassicicola, A. raphani and A. alternata*). The eco-friendly management approaches for *Alternaria* diseases (including the use of resistant cultivars, botanical extracts, cultural, chemical and biological control measures) are discussed.

Khodaei and Arzanlou (2013) reported a total number of 97 fungal isolates recovered from sunflower leaves with leaf spot disease symptoms from the sunflower fields in northwestern zone of Iran. All of the isolates were identified as *Alternaria alternata* based on cultural and morphological characteristics.

Hajipour *et al.,* (2014) studied the *Alternaria* species from potato fields of West Azerbaijan province, foliage and stems that had suspected

infections with *Alternaria* fungi were collected. Totally, 141 isolates belonging to the genus *Alternaria* were isolated and purified. Pathogenicity studies on isolates of identified species were done on potato cultivar Algria and their pathogenicity were confirmed based on Koch´s postulates. All the studied isolates were pathogenic, although the degree of pathogenicity based on diameter of necrotic area varied among different species.

2.3 Efficacy of bio-control agents (*in vitro* and *in vivo*)

Morshed (1985) noticed that the fungus *Trichoderma viride* was effective in checking the growth of *A. tenuis* (*A. alternata*) in dual culture. The culture filtrate of *Aspergillus* and *Trichoderma viride* were effective to retard the growth of *A. alternata*.

Amaresh (2000) reported that among fungi *T. viride* and *T. harzianum* overgrew and inhibited the growth of *A. helianthi* while the bacterium *P. fluorescens* produced maximum inhibition zone.

Babu *et al.,* (2000) evaluated the efficacy of six *Trichoderma* species on early blight of tomato. Among the six species, *T. harzianum* exerted the highest inhibition of the mycelial growth (50.22%) of the pathogen over control fallowed by *T. viride.*

Kota (2003) described that *Trichoderma harzianum* and *T. virens* highly inhibited the growth of *A. alternata* under *in vitro* condition. The highest inhibition of mycelial growth of *A. alternata* was observed in ten per cent chromalaena leaf extract, while garlic bulb extract at 10 per cent gave highest inhibition of spore germination of the same fungus.

Sanjeetkumar *et al.,* (2005) observed that in dual culture, all the three antagonists *viz. Trichoderma virens, T. harzianum and T. viride* overgrew the colony of *Alternaria alternata* but *T. viride* parasitized the test fungus earliest. Studies on hyphal interaction between antagonist and test

fungus revealed disorganization of protoplasmic content and lysis of host hyphae.

Singh *et al.,* (2005) studied the efficacy of *Trichoderma viride, T. harzianum, T. hamatum, T. koningii* and *Pseudomonas fluorescens* in controlling *Alternaria brassicae* under laboratory conditions. All the fungal antagonists inhibited the growth of *A. brassicae*, with *T. viride* recording the highest growth inhibition of the pathogen (67.9%).

Rao (2006) tested that six bio agents for their efficacy against *A. helianthi* by dual culture technique, maximum inhibition of mycelial growth was noticed in *Pseudomonas fluorescens* (73.74%), which was found at par with *T. harzianum* (71.52%). Among the six bio agents tested for their efficacy in overcoming seed-borne infections of *A. helianthi* and other fungal contaminations by rolled towel method, seed treatment with *P. fluorescens* showed least seed infection with maximum per cent germination and vigour index. *P. fluorescens* seed treatment at 0.8 per cent concentration was found to be at par with *P. fluorescens* seed treatment at 0.6 per cent concentration with respect to per cent seed infection and per cent seed germination; however, they differed significantly with respect to vigour index. Among the test antagonists, *T. viride* was most effective in inhibiting the growth of *A. porri*. This was followed by native isolate of *T. harzianum, T. koningii* and *T. virens*. In case of *A. alternata*, out of 11 antagonists tested, *T. harzianum* (native isolate) recorded highest inhibition of radial growth. *T. koningii, T. viride, T. harzianum* (IABT), *T. harzianum* (UASD) and *T. virens* were next in order.

Arunkumar (2008) noted that among six bio control agents tested against *A. alternata* under laboratory condition in dual culture, *T. harzianum* recorded highest inhibition of radial growth where as *T. koningii, T. viridae,* and *T. virens* were next in order. The bacterial antagonists proved least effective.

Dalpati *et al.,* (2010) evaluated four different bio-agents (*T. harzianum, T. viride, P. fluorescens* and *Bacillus subtilis*) against the *Alternaria macrospora* causing leaf spot of cotton *in vitro*. Among the four bioagents *T. harzianum* was found superior as compared to others as it inhibited the growth by 76.66 per cent followed by *Bacillus subtilis* (73.66%).

Chethana *et al.,* (2012) studied five biocontrol agents (*Trichoderma harzianum, Chaetomium* sp. *Beauveria bassiana, Verticillium lecanii* and *Metarhizium anisopliae*) under *in vitro*. Among the biocontrol agents evaluated, maximum inhibition (79.5%) was recorded in *T. harzianum.*

Apet *et al*., (2014) reported that among the bioagents tested, significantly highest mycelial growth inhibition was recorded with *T. viride* (86.67%), followed by *T. hamatum* (78.34%), *T. koningii* (76.67%), *T. lignorun* (68.15%), *T. harzianum* (53.16%).

Joshi *et al.,* (2014) evaluated two species of *Trichoderma* against *Alternaria alternata* incited leaf spot and fruit rot of papaya. However, *T. viride* (S.K. Nagar) showed significantly maximum per cent growth inhibition (91.95%)

Kamal *et al.,* (2014) found four isolates of *T. harzianum* and five isolates of *T. longibrachiatum*. The antagonistic activity of these isolates against onion purple blotch pathogen *Alternari porri* was studied *in vitro* using dual culture assay. *T. harzianum* exhibited high ability to compete on potato dextrose agar (PDA) medium causing the maximum rate of pathogen inhibition (73.12%), while isolates of *T. longibrachiatum* showed inhibition rate equalling 70.3 per cent. Application of *T. harzianum* to control purple blotch disease *in vivo* under greenhouse conditions caused disease reduction up to 52.3 and 79.9 per cent before and after 48 hours of pathogen inoculation, respectively.

Barman *et al.,* (2015) evaluated seven different antagonistic micro organisms *viz., Trichoderma viride, T. virens, T. harzianum, T. longibrachiatum, T. reesi, B. subtilis* and *P. fluorescens* on leaf blight of tomato caused by *Alternaria alternata*. Among the different antagonistic agents, *T. viride* showed 71.9 per cent inhibition of mycellial growth over control.

Brahmane (2015) observed efficacy of bio-agents against *Alternaria porri* causing purple blotch of onion. The bioagent *T. harzarium* was found most effective in controlling the pathogen. Lowest disease severity (37.67%) was recorded in the spray treatment with *T. harzarium.* It was followed by *T. viride.*

Chavan *et al.,* (2015) recorded bioagents *viz., T. viride, T. harzianum* and *T. longibrachiatum* significantly highest inhibitions of 81.49, 76.50 and 73.97 per cent, respectively against *Alternaria brassicae* of cauliflower.

Falake *et al.,* (2015) tested biocontrol agent namely, *T. viride*, *Gliocladium* spp., *T. harzianum*, *T. koningii* and *P. fluorescens* for their efficacy against *A. solani in vitro*. Percent inhibition of the test pathogen ranged from 45.16 to 75.67 per cent. *T. harzianum*, was found to be the most effective antagonist which caused growth inhibition of 75.57 per cent followed by *T. viride* (64.28%).

Koley *et al.,* (2015) studied on growth inhibition of fungus *A. solani* causing early leaf blight of tomato *in vitro* for determining the efficacy of six bio-control agents and 12 botanicals. *T. harzianum* and two isolates of *T. viride* resulted less than 40 per cent growth inhibition.

Ramanujam *et al.,* (2015) showed that fungal and bacterial antagonists were evaluated against early blight pathogen of tomato, *Alternaria solani* under *in vitro*, glasshouse and field conditions. Among the isolates tested, *T. harzianum* showed significant inhibition of *A. solani* under

in vitro (72.78%) and glasshouse conditions (62.60%). Seedling dip and foliar applications of *T. harzianum*, *T. viride* and *P. fluorescens* decreased the early blight incidence up to 62 per cent and increased tomato yield up to 37 per cent over control in field trials.

2.4 Efficacy of botanicals (*in vitro* and *in vivo*)

Khanna and Chandra (1972) found that leaf extracts of *Azadirachta indica* inhibited spore germination of *Alternaria alternata* isolated from wheat.

Meena and Mariappan (1993) reported that leaf extracts of *Azadirachta indica, Mentha arvensis, Catharanthus roseus, Lantana camara, Pongamis pinnata, Vitex negundo* and *Nerium odorum (Nerium olender*) and flower extracts of *Catharanthus roseus* inhibited mycelial growth and spore germination of the seed borne mycoflora of sorghum including *Alternaria tenuis, Alternaria alternata, Aspergillus flavus, Curvularia lunata (Cochlibolus lunatus)* and *Fusarium moniliforme (Gibbrella fusikuroi)*. The neem extracts and those of *Catharanthus roseus* and *Lantana camara* were found more effective than other plant extracts tested.

Datar (1996) showed the inhibitory effect of garlic clove extract on the mycelial growth of *A. tenuis* - causal organism of brinjal leaf spot.

Rashmi and Yadav (1999) reported all the plant extracts less effective at lower concentrations. There was a positive correlation between concentration and growth inhibition percentage. The bulb extracts of *A. sativum* and leaf extracts of *Ocimum basilicum* L. at two per cent were most effective in suppressing radial growth and biomass production of *A. alternata.*

Dubey (2001) evaluated five plant extracts against leaf spot and flower blight of marigold (*Alternaria tagetica*). Among the plant extracts / products, the neem leaf extract @ 10 per cent was found most effective under field condition and controlled the disease by 47.3 per cent and 31.83 per cent on leaf and flower, respectively and increased the flower yield by 48.7 per cent.

Patni *et al.,* (2005) tested methanol extracts of six medicinal plants (*Azadirachta indica, Parthenium hysterophorus, Calotropis procera, Datura alba, Eucalyptus globulus and Polyalthia longifolia*) against *Alternaria* blight (*Alternaria brassicae*) of Indian mustard. Eucalyptus, Ashok and Calotropis extracts, in that order, were promising in limiting the growth and sporulation of the pathogen.

Rao (2006) studied twelve plant extracts against *A. helianthi* by poison food technique *Allium sativum* showed maximum inhibition of mycelial growth (67.77%) followed by *Azadirachta indica* (60.68%). *A. sativum* showed significant increase in inhibition of mycelial growth at 10 per cent concentration (70.73%) compared to five per cent concentration (64.81%) and also differed significantly from *Azadirachta indica* at 10 per cent concentration (61.10%).of the twelve plant extracts tested against seed-borne infection of *A. helianthi* and other fungal contaminations by rolled towel method, *A. indica* was found to be superior, followed by *A. sativum. A. indica* showed seed infection of 25 per cent and vigour index of 1145 at 10 per cent concentration which was found on par at 5 per cent concentration (26.0 and 1125) but differed significantly with respect to seed germination at 10 per cent concentration (76.5%) over 5 per cent concentration (74%).

Sharma *et al.,* (2007) reported that the neem leaf extract showed high efficacy to inhibit the radial growth of *A. solani* (43.3 and 26.7% at 0.1% and 0.01%, respectively).

Suwitchayanon and Kunasakdakul (2009) tested clove (*Syzygium aromaticum* (Linn.) Merr. & Perry) and turmeric (*Curcuma longa* L.,) extracts against crucifer pathogens using soaking method. Both extracts showed inhibitory effects on the pathogens. The results of antifungal activities revealed that clove extract was indicated the minimum inhibitory concentration (MIC) of *Alternaria brassicicola* and *Fusarium oxyporum* at 1900 ppm and 2300 ppm respectively.

Dalpati *et al.,* (2010) evaluated ten botanicals *viz*. neem, custard apple, lantana, eucalyptus, marigold, tamarind, kanher, garlic, datura and congress grass against the *Alternaria macrospora* causing leaf spot of cotton *in vitro*. The per cent inhibition of botanicals ranged from 44.59 to 8.25 per cent. Lantana and datura were found effective as it restricted 44.59 and 30.88 per cent respectively. Botanicals like tamarinds found least effective as it prohibited the growth by 8.25 per cent only.

Anamika and Sobita Simon (2011) evaluated the efficacy of plant extracts at 5 and 10 per cent concentration on *Alternaria alternata* causes dry rot of *aloe vera*. Neem leaf extract gave 58.6 per cent inhibition of radial growth followed by *Oscimum sanctum* L. which gave 54.7 per cent inhibition.

Chethana *et al.,* (2012) studied bio efficacy of six plant products (Clerodendron, cinnamon, garlic, neem oil, pongamia oil and turmeric) were evaluated under *in vitro.* Among plant products evaluated, fresh aqueous extract of garlic (20%) was effective in causing 100 per cent inhibition of mycelial growth. Neem oil and pongamia oil (20%) caused 76.94 and 69.94 per cent inhibition.

Sasode *et al.,* (2012) studied the effectiveness of some botanicals against *A. brassicae* and *A. brassicicola*. Among the crude extract 10 per cent the minimum growth was recorded in neem followed by eucalyptus,

tulsi, lantana, datura and pudina. Neem was significantly superior over tulsi, lantana, datura and pudina but at par with eucalyptus.

Kavita and Dalbeer (2013) evaluated the different botanicals against *Alternaria brassicae* in *in vitro* condition. The present study aims to evaluate the effectiveness of 46 botanicals extract *viz., Piper nigrum* L. (seed), exhibited between 85.33 to 84.44 per cent, *Ocimum sanctum* and *Anagallis arvensis*) exhibited 63.71 to 60.61 per cent, and Eucalyptus (Leaf*), Lantana camara* (Leaf), *Allium sativum* (Bulb) are exhibited in between 49.67 to 41.19 per cent, @ against 10 per cent concentration *Alternaria brassicae* condition by poisoned food technique.

Apet *et al*., (2014) tested aqueous extracts of some botanicals tested (@ 10 and 20 %) against *Alternaria alternata*. However, significantly highest average mycelial growth inhibition was recorded with *A. sativum* (74.45%).

Singh *et al.,* (2014) reported methanolic extracts of six selected plant leaves *viz., Parthenium hysterophorus, Vernonia amygdalina, Eucalyptus camaldulensis, Nerium oleander, Lantana camara , Ocimum sanctum* etc. were *in-vitro* screened for their antifungal activity against. *A. alternata* at 5, 10 and 20 per cent concentration. Highest reduction in mycelial growth was achieved by Parthenium, Ocimum, Lantana, Vernonia and Eucalyptus.

Harde and Suryawanshi (2014) evaluated aqueous extracts of 13 botanicals / plant species *in vitro* (@10, 20 %) against *Alternaria brassicae*, inciting *Alternaria* blight of mustard (*Brassica juncea* L.). Among the botanicals evaluated, *Azadirachta indica* (Neem) was found most fungi toxic and recorded significantly highest mean growth inhibition (80. 46%) of the test pathogen. This was followed by the botanicals *viz., Oscimum sanctum* (Tulsi) (71.41%), *Lantana camera* (65.65%), *Eucalyptus globulus* (53.38%), *Allium sativum* (32. 55%).

Joshi *et al.,* (2014) described efficacy of plant extracts, biological agents and fungicides against *Alternaria alternata* incited leaf spot and fruit rot of papaya. The extract of garlic clove (*Allium sativum*) at 15 per cent concentration showed maximum growth inhibition of *A. alternata* (88.91%) which was at par with lantana leaves (88.35%) and datura leaves (87.51%).

Kantwa *et al.,* (2014) tested efficacy of seven plant extracts, each at five concentrations (50, 100, 200, 500 and 1000 ppm), against the mycelial growth and sporulation of *Alternaria alternata*. Irrespective of the concentration, garlic clove extract was found most effective in inhibiting the mycelial growth (46.60 %) of *A. alternata* followed by neem (43.30%) and datura (40.30%) leaf extract.

Sobhy *et al.*, (2014) noted the efficacy of certain plant extracts against the *Alternaria porri* antifungal activity of some aqueous plant extracts (*Azadirachta indica, Cydonia oblonga, Datura stramonium, Eucalyptus globulus, Foeniculum vulgare, Ocimum basilicum, Rosmarinus officinalis and Salix mucronata*) was assayed *in vitro* by dry weight technique. The data indicated that significant effect of *A. indica* and *D. stramonium* extracts on fungal growth of *A. porri*. Under greenhouse conditions, application of the aqueous extract of *A. indica* produced the highest reduction in disease severity comprising 70 per cent.

Brahmane (2015) observed efficacy of plant extract against *Alternaria porri* causing purple blotch of onion. Among the plant extract 5 per cent neem seed kernel extract gave maximum control of pathogen i.e. 44.24 per cent over control, followed by followed by *Mentha arvensis, Allium sativum, Zingiber officinale, Vitexne gundo.*

Chavan *et al.,* (2015) tested botanicals against *Alternaria brassicae* of cauliflower. Botanicals *viz., A. sativum, A. indica* and

Z. officinale causes significantly highest mean mycelial inhibition of 85.62, 80.50 and 68.93 per cent, respectively.

Falake *et al.,* (2015) evaluated 13 plant extracts which were locally available *in vitro* against *A. solani* and were tested by poisoned food technique. Overall percent inhibition of growth of the fungus ranged from 16.05 to 80.46 per cent. *Azardicata indica* recorded maximum average inhibition of 80.46 per cent followed *O. sanctum* 71.41 per cent. Kansara and Sabalparae (2015) evaluated *in vitro* various botanicals against *Alternaria alternata* of niger. Aqueous extracts of all the botanicals tested (@ 10 %) were antifungal to the test pathogen. Among the effective botanicals, highest average mycelial growth inhibition was recorded with bulb extract of garlic (67.30%) followed by leaf extract of eucalyptus (60.08%), neem (56.28%).

Koley *et al.,* (2015) studied on growth inhibition of fungus *A. solani* causing early leaf blight of tomato *in vitro* for determining the efficacy of botanicals. The aqueous leaf extract of *Datura stramonium* was the best followed by *Azadirachta indica* oil and *Lantana camara* leaf extract showing growth inhibition 57.03 per cent, 51.35 per cent and 48.02 per cent of the fungus, respectively. The efficiency of the botanicals was significantly the highest at 15 per cent concentration for all the botanicals than 5 and 10 per cent.

Ariafar and Zacharia (2016) tested botanicals against early blight disease of potato plant which is caused by the fungus, *Alternaria solani*. The antifungal activity of five plants extracts namely *Azadirachta indica* (neem), *Eucalyptus chamadulonsis* (eucalyptus), *Ocimum tenuiflorum* (tulsi), *Nerium oleander* (kaner), and *Calotropi sprocera* (madar) at 10 per cent concentration and carbendazim 0.1 per cent concentration as a check, was

tested for management of *Alternaria solani in vitro* and *in vivo*. In the present investigation the highest inhibition of mycelial growth of *Alternaria solani* was achieved by fresh aqueous extract of *Eucalyptus chamadulonsis, Ocimum tenuiflorum, and Azadirachta indica* caused the highest reduction of mycelial growth of *A. solani* (57.35, 50.0 and 44.12%, respectively), while *Nerium oleander*, and *Calotropi sprocera* showed the lowest inhibition of mycelial growth of the pathogen as compare to treated check and untreated check.

2.5 Varietal screening

Robinson (1957) studied symptoms and methods for screening of carnation cultivars against disease caused by *Alternaria dianthi*.

Yadav *et al.,* (1999) showed that screening of Indian mustard germplasm for resistance to *Alternaria* blight and white rust.

Thammaiah *et al.,* (2004) evaluated chrysanthemum cultivars against *Alternaria* leaf spot disease under natural conditions. There were twelve cultivars *viz.*, Chandini, Bangalore, Raja, Dundi, Sarval, Co-1, No- 9, Indira, Karnool, No-5, Co-2, and Gloden Red. Among twelve cultivars, cultivar Indira recorded the lowest *Alternaria* leaf spot index (2.37%) followed by Co-2 (3.06%) and Co-1 (6.49). The highest *Alternaria* leaf spot index was recorded in the cultivar Chandini (39.12%) followed by Raja (38.67%). Cultivar Indira, Co-2 and Co-1 can be used as a resistant source.

Kushal *et al.,* (2015) noted screening of onion genotypes against purple blotch caused by *Alternaria porri*. The study was conducted to identify the tolerant/ resistant onion varieties purple blotch disease caused by *A. porri* with high yield potential for two seasons.

3. MATERIAL AND METHODS

The studies on leaf spot disease of carnation were carried out at the College of Agriculture, Pune - 05 during 2015 - 2017. The details of the material used and methods followed during the course of present investigation are given below.

3.1 Material

3.1.1 Collection of leaf spot disease samples

The samples of leaf spot of carnation plants were collected from National Agricultural Research Project, Ganeshkhind, Pune – 411 007 and subjected to isolation of the pathogen on PDA.

3.1.2 Glasswares

The standard Borosil brand glasswares used for laboratory study were Petri plates, test tubes, moisture chambers (Desiccators), beakers, conical flasks, measuring cylinders, slides, pipette, spirit jar etc.

3.1.3 Equipments

Various laboratory equipments used during the study were Autoclave, Laminar air flow cabinet, B.O.D. incubator, Hot air oven, Microscope, Refrigerator, pH meter, Shaker, Top pan balance etc.

3.1.4 Culture medium

The common laboratory culture medium Potato dextrose agar (PDA) was used for isolation of the pathogen responsible for causing leaf spot disease of carnation and maintained its pure culture on PDA slant tubes in refrigerator for further study. PDA was also used for dual culture technique and poison food technique.

3.1.5 Miscellaneous material

Inoculation needles, forceps, cork borer, scissors, scalpel, earthen pots, cover slips, micro slides, spirit lamp, cotton, mercuric chloride, polyethylene bags, blotting papers, glass rods, labels, stickers, glass marking pencils, mortar and pestle, sodium hypochlorite, mixer-cumgrinder, distilled water, sterilized water, scale, packing boxes, test tube stand etc. available in the laboratory of Plant Pathology Section were used during present studies.

3.1.6 Bio-agents

Biocontrol agents *viz., Trichoderma viride, T. harzianum, T. hamatum, T. virens* and *T. koningii* were obtained from Biological Nitrogen Fixation Scheme, College of Agriculture, Pune - 05.

3.1.7 Botanicals

Aqueous extracts (each @ 10%) of Neem leaf, Garlic clove, Onion bulb, Nilgiri leaf, Tulsi leaf, Ghaneri leaf, Clove, Kaner leaf were used for *in vitro* and/or *in vivo* studies.

3.2 Methods

3.2.1 Collection of leaf spot disease samples

Leaf spot diseased samples of carnation plants were collected from the National Agricultural Research Project, Ganeshkhind, Pune - 411 007. The spoted leaves were collected in separate sterile paper bags to avoid contamination and were brought to the laboratory for further investigations.

3.2.2 Culture medium

The basic culture medium potato dextrose agar (PDA), comprising following ingredients was prepared.

Peeled potatoes : 200 g

Dextrose : 20g

Agar agar : 20g

Distilled water : 1000ml

For the purpose, 200 g peeled and sliced potatoes were boiled in one liter of water until potatoes became soft. Then it was filtered through muslin cloth and adjusted its final volume to one litre. To this dextrose 20 g and agar agar 20 g were added, boiled again, filled into glass conical flasks (250 ml cap), plugged with non-absorbent cotton and sterilized in an Autoclave at 1.54 kg/cm^2 pressure and corresponding 121°C temperature, for 15 minutes.

3.2.3 Isolation and purification of the Pathogen

The leaf spot diseased leaf of carnation plants were first washed in tap water and cut into small pieces, surface sterilized in 0.1 per cent mercuric chloride solution for 1½ min. and washed sequentially in sterile water to remove traces of $HgCl_2$. Two to three disinfected leaf bits were then inoculated at equidistance on each PDA plate and incubated at room temperature (27 ± 1°C) for 7 days. The fungal growth was transferred to PDA slant tubes by hyphal tip isolation method, by frequent sub-culturing, fungus was purified and pure culture was maintained on PDA slant tubes for further studies.

3.2.4 Pathogenicity test

Carnation seedlings (Manuela) were transplanted in earthen pots filled with sterilized soil. Six days after transplanting seedling were sprayed with sterile distilled water before inoculation. Then plants were covered with a polythene bag for 24 hours. Spore and mycelial suspension was prepared in

sterile water from nine-day-old culture. The spores suspension was sprayed and swabbed with moist cotton on the leaves. Such inoculated plants were again covered with polythene bag. After 48 hours of incubation, polythene bag was removed and the plants were kept in screenhouse. Control was maintained by spraying the plants with only sterile distilled water. Observations were made for symptoms development periodically. Reisolations were made from infected plants and the cultures thus obtained were compared with original cultures to confirm the pathogenicity of pathogen.

3.2.5 Identification of the Pathogen

The isolated fungus was identified as *Alternaria dianthi* on the basis of morphological characters by referring standard book in mycology and description of morphology given by Seung-Hun *et al.,* (1989).

3.2.6 Disease management study

3.2.6.1 *In vitro* evaluation of bio-agents

Antagonistic effect of four bio-agents (as under treatment details) against *Alternaria dianthi* was studied by adopting dual culture technique (Dennis and Webster, 1971).

Autoclaved and cooled (40°C) PDA medium was poured (@ 20 ml/plate) in to sterilized glass petri plate and allowed to solidify. Upon solidification of PDA, these plates were aseptically inoculated with two mycelial discs (each 5 mm), one each of the test fungal bio agent and test pathogen, by placing exactly opposite to each other and about 1 cm away from the edge of the plate. In case of bacterial antagonist, it was streaked at opposite side of the test fungus. The five mm mycelial disc placed at centre of the petri plate without any bio-agent served as control. Both treated and

untreated control plates were incubated for 7 days at 27 ± 1°C. After 7 days of incubation, the linear growth of the pathogen was measured.

The experiment was planned with Completely Randomized Design with four replications and six treatments.

Treatments details:

T_1 : *Trichoderma viride*

T_2 : *T. harzianum*

T_3 : *T. hamatum*

T_4 : *T. virens*

T_5 : *T. koningii*

T_6 : Control

Observations on radial mycelial growth/colony diameter of the *Alternaria dianthi* were recorded when untreated control plate was fully covered with mycelial growth of the test pathogen.

Per cent mycelial growth inhibition of the *A. dianthi* with treatments over untreated control was calculated by applying following formula (Vincent, 1927).

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

Where, I = Per cent Inhibition of fungal growth.

C = Growth/colony diameter (mm) of the fungus in control plate.

T = Growth/colony diameter (mm) of the fungus in treatment plate.

3.2.6.2 *In vitro* evaluation of botanicals

Preparation of plant extracts

Ten gram of plant parts was weighed accurately and washed thoroughly under the tap water. The plant material was then made into a paste using mortar and pestle by adding 10 ml of sterilized distilled water. The crude material was then filtered through a double layered muslin cloth and filter paper (Whatman No. 1) under aseptic condition. The filtrate thus obtained was used in the experiments.

The eight aqueous extracts *viz*., Neem leaf, Garlic clove, Onion bulb, Nilgiri leaf, Tulsi leaf, Ghaneri leaf, Clove and Kaner leaf (each @ 10% w/v) were obtained by above mentioned method. This aqueous extracts of botanicals were evaluated *in vitro* by adopting poison food technique (Nene and Thapliyal, 1982), using PDA as basal medium. Autoclaved and cooled (40°C) PDA medium was dispensed @ 100 ml per glass conical flask (250 ml cap). To these flasks, based on active ingredients, test aqueous extracts were added separately and shaken vigorously to ensure their uniform mixing in the medium. This PDA medium amended separately with the aqueous extract of the botanicals were poured aseptically in sterilized glass petri plates (90 mm diam.) and allowed to solidify. Upon solidification, these plates were inoculated with 5 mm disk obtained from a week old pure culture of *Alternaria dianthi*. Plain PDA plated (without any botanical) and inoculated with a mycelial disc (5 mm) of the test pathogen was kept as untreated control. The experiment was replicated three times. Both treated and untreated PDA plates were incubated at 27±1°C, in BOD incubator for a week.

The experiment was planned with Completely Randomized Design and all nine treatments replicated thrice.

Treatments details:

T_1	:	Neem leaf extract	-	10 per cent
T_2	:	Garlic clove extract	-	10 per cent
T_3	:	Onion bulb extract	-	10 per cent
T_4	:	Nilgiri leaf extract	-	10 per cent
T_5	:	Tulsi leaf extract	-	10 per cent
T_6	:	Ghaneri leaf extract	-	10 per cent
T_7	:	Clove extract	-	10 per cent
T_8	:	Kaner leaf extract	-	10 per cent
T_9	:	Control		

Per cent mycelial growth inhibition of the test pathogen, over untreated control was calculated by applying the formula (Vincent, 1927) as detailed under sub-heading 3.2.6.1, in this chapter.

3.2.6.3 *In vivo* evaluation of effective bio-agents and botanicals

Most effective two bio-agents and three botanicals were evaluated in pot culture for the management of leaf spot disease in carnation. The experiment was conducted in the earthen pots, by leaf inoculation method under screenhouse conditions at Plant Pathology Section, College of Agriculture, Pune.

Experimental details:

Design : Completely Randomized Design

Variety : Manuela

Replications : 4

Treatments : 6

Treatment details

T_1 : *Trichoderma viride* (0.5%)

T_2 : *T. hamatum* (0.5%)

T_3 : Neem leaf extract (10%)

T_4 : Garlic clove extract (10%)

T_5 : Clove extract (10%)

T_6 : Control

Six day after transplanting, carnation seedlings were inoculated by *A. dianthi* using leaf inoculation method. In sterile water, suspension of the test bio-agent (each @ 0.5% w/v) and the botanicals (each @ 10% w/v) used in the present studies at their recommended dose were separately sprayed twice i.e. seven and fifteen days after the appearance of disease. Pot sprayed with plain water served as check. Four pots per treatments per replication were maintained. The data on leaf spot intensity was recorded before spray and 7 days after spray using 0-9 rating scale (Datar and Mayee, 1986) as mention in 3.2.7.

$$\text{Per cent disease intensity (PDI)} = \frac{\text{Sum of all numerical ratings}}{\text{No. of leaves assessed x Maximum disease rating}} \times 100$$

$$\text{Per cent disease control (PDC)} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

Where details on,

PDI = Per cent disease intensity

3.2.7 Varietal Screening

Eight seedlings of ten varieties each transplanted in the earthen pots filled with sterilized soil. Six days after transplanting seedling were sprayed with sterile distilled water before inoculation. Then plants covered with a polythene bag for 24 hours. Spore suspension was prepared in sterile water from nine-day-old culture. The spores suspension was sprayed and swabbed with moist cotton on to leaves. Such inoculated plants were again covered with polythene bag. After 48 hours of incubation polythene bag was removed and the plants were kept in screen-house. Following ten varieties of carnation were screened and observations on leaf spot intensity were recorded at 15 days interval upto 60 days after disease incidences. Per cent leaf spot intensity was calculated as detailed under 3.2.6.3, in this chapter.

Varieties of carnation

1. Irene
2. Gaudina
3. Bizet
4. Eskimo
5. Amado
6. Gwen
7. Ambrose
8. Orange viana
9. White liberty
10. Manuela

These carnation varieties screened were categorized by using following disease reaction scale (Datar and Mayee, 1986).

Grade	Per cent leaf area affected	Disease Reaction
0	No visible symptom	Highly resistant (HR)
1	0-1	Resistant (R)
3	1.1-10	Moderately resistant (MR)
5	10.1-25	Moderately susceptible (MS)
7	25.1-50	Susceptible (S)
9	>50	Highly susceptible (HS)

3.2.8 Statistical analysis

Before analyzing the data, per cent values were converted into arcsine values. The data on disease intensity and per cent disease control were analyzed. Standard error (SE) and critical difference (CD) at 5 per cent level of significance were worked out (Panse and Sukhatme, 1967). The treatment means were compared at 5 per cent level of significance.

4. RESULTS

The present research work on leaf spot disease of carnation with respect to isolation, identification, *in vitro* and *in vivo* evaluation of different botanicals and bio-agents & varietal screening against leaf spot (*Alternaria dianthi*) of carnation was carried out. The results on these aspects are presented here as under.

4.1 Isolation and pathogenicity test

4.1.1 Isolation and purification

Leaf spot affected samples of carnation plants were collected from National Agricultural Research Project, Ganeshkhind, Pune - 411 007.

Tissue isolation of leaf spot of carnation yielded light grey to blackish coloured colonies on PDA within 3 - 4 days of incubation. Pure culture of the test pathogen was obtained by hyphal tip method and maintained on PDA slant for further studies (Plate - 1)

4.1.2 Pathogenicity

The pathogenicity of the test pathogen attempted by leaf inoculation method resulted in expression of typical leaf spot symptoms on carnation seedlings in pot culture under screen-house conditions. The leaf spot symptoms induced were similar to naturally occurring leaf spot disease on carnation plants (Plate – 3 and 4).

For further studies, isolations were made from the artificially inoculated plant which depicted same symptoms as described earlier. The fungus isolated by reisolation was identical with the original culture of *A. dianthi* in all respect and the pathogenicity and Koch’s postulates were proved successfully for the isolate.

4.1.3 Identification of the pathogen

Temporary mounts of the pure culture of the test pathogen and observations under research microscope revealed that the mycelium of the fungus as septate and profusely branched. Condia existed (Plate - 2). The conidiophores measured 42.26 µm in length and 4.29 µm in width. They were light olivaceous to dark brown in colour, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal and 2-10 transverse septa.

The isolated fungus was identified as *Alternaria dianthi* on the basis of morphological characters by referring standard book in mycology and description of morphology given by Seung-Hun *et al.,* (1989).

4.2 Symptomatology

In pathogenicity test of *Alternaria dianthi* at early stage of disease development, small irregular to circular dark brown spots were observed on lower leaves of carnation. Concentric rings formed as a result of irregular growth pattern by the organism in the leaf tissue.

4.3 *In vitro* evaluation of bio-agents

1The competitive ability of antagonists against *Alternaria dianthi* was studied by dual culture technique and the results obtained are presented in the Table 1, shown in Plate 5 and depicted graphically in fig. 1 and 2.

The result revealed that all the bio-agents showed significant reduction in the growth of pathogen than the control. Minimum growth of pathogen was observed in the *Trichoderma viride* (23.75mm) and it was on par with *T. hamatum* (24.50mm). The rest of treatments ranged between 32.25 to 36.00mm. The treatments *T. koningii*, *T. virens* and *T. harzianum* were at par with each other. Control showed 88.00 mm pathogen growth.

The per cent inhibition was highest in the treatment *T. viride* (73.01) and it was immediately followed by *T. hamatum* (72.15). Least per cent inhibition showed by *T. koningii*, *T. virens* and *T. harzianum* were 63.35, 60.79 and 59.09, respectively.

Table1. *In vitro* efficacy of bio-agents against *Alternaria dianthi*

Treatment No.	Treatments	Mean colony diameter of pathogen(mm)*	Per cent inhibition
T_1	*Trichoderma viride*	23.75	73.01
T_2	*Trichoderma harzianum*	36.00	59.09
T_3	*Trichoderma hamatum*	24.50	72.15
T_4	*Trichoderma virens*	34.50	60.79
T_5	*Trichoderma koningii*	32.25	63.35
T_6	Control	88.00	-
S.E. (m) ±		0.94	
C.D. (0.05)		2.79	

* = Mean of four replications

4.4 *In vitro* evaluation of botanicals

The results obtained are presented in Table 2, shown in plate 6 and depicted graphically in fig. 3 and 4.

The results revealed that all the botanicals showed significant reduction in the growth of pathogen than the control. Minimum growth of pathogen was observed in the clove extract (6.00 mm) which was significantly superior over all treatments. The rest of treatments ranged between 31.33 to 47.00 mm. The treatments Neem leaf exatract (31.33 mm) followed by Garlic clove extract (36.00mm). Tulsi leaf extract (42.67 mm), Ghaneri leaf extract (44.66 mm) and Nilgiri leaf extract (45.00 mm) were at par to each other. Control showed 71.33 mm pathogen growth.

The per cent inhibition was highest showed in clove extract (91.58), which is superior over all treatments. The rest of treatment ranged between 34.10 to 56.07 per cent. Per cent inhibition in Neem leaf exatract (56.07) followed by Garlic clove extract (49.53). Tulsi leaf extract (40.17), Ghaneri leaf extract (37.37) and Nilgiri leaf extract (36.91) were at par to each other.

Table 2. *In vitro* efficacy of botanicals @ 10 per cent against *Alternaria dianthi.*

Treatment No.	Treatments	Scientific name	Mean colony diameter * (mm)	Per cent inhibition
T_1	Neem leaf extract	*Azadirachta indic* A.Juss.	31.33	56.07
T_2	Garlic clove extract	*Allium sativum* L.,	36.00	49.53
T_3	Onion bulb extract	*Allium cepa* L.,	46.67	34.57
T_4	Nilgiri leaf extract	*Eucalyptu globulus* Labill.	45.00	36.91
T_5	Tulsi leaf extract	*Ocimum basilicum* L.,	42.67	40.17
T_6	Ghaneri leaf extract	*Lantana camara* L.,	44.67	37.37
T_7	Clove extract	*Syzygium aromaticum* (Linn.)Merr.&Perry.	6.00	91.58
T_8	Kaner leaf extract	*Nerium oleander* L.,	47.00	34.10
T_9	Control	-	71.33	-
S.E. (m)±			1.12	
C.D. (0.05)			3.31	

* = Mean of three replications.

4.5 *In vivo* evaluation of effective bio-agents and botanicals

The most effective two bio-agents *viz.*, *T. viride* (0.5%) and *T. hamatum* (0.5%) and three botanicals *viz.*, aqueous extracts of Clove (10%), Neem leaf (10%) and Garlic clove (10%) were integrated for management of carnation leaf spot in pot culture under screen-house conditions and results are presented in Table 3, shown in plate 7 and depicted graphically in fig. 5 and 6.

The result revealed that, the leaf spot intensity varied from 7.63 to 76.38 per cent. Minimum leaf spot intensity was observed in Clove extract (7.63%), which was superior over all treatments. The rest of treatments ranged between 26.04 to 42.00 per cent. Control revealed 76.38 per cent leaf spot intensity.

The highest per cent disease control was recorded in Clove extract (90.01), followed by Neem leaf extract (65.90) and Garlic clove extract (62.63). Least per cent disease control was showed by *Trichoderma viride* and *T. hamatum* 52.53 and 44.99, respectively.

Table 3. *In vivo* evaluation of effective bio-agents and botanicals against *Alternaria dianthi*.

Treatment No.	Treatments	Conc. used (%)	Per cent leaf spot intensity*	Per cent disease control
T_1	*Trichoderma viride* (6×10^7 spores/g)	0.5	36.25 (35.36)	52.53
T_2	*Trichoderma hamatum* (7×10^7 spores/g)	0.5	42.00 (40.57)	44.99
T_3	Neem leaf extract	10	26.04 (32.02)	65.90
T_4	Garlic clove extract	10	28.46 (29.44)	62.63
T_5	Clove extract	10	7.63 (15.63)	90.01
T_6	Control	-	76.38 (61.15)	-
S.E. (m) ±			3.18	
C.D. (0.05)			9.43	

* = Mean of four replications.

Figures in the parentheses are arc sin transformed values.

4.6 Varietal screening and reactions

A total of ten carnation cultivars were tested for their reactions against leaf spot of carnation, under screen house conditions and results obtained are presented in the Table 4 and shown in Plate 8.

The result revealed that the per cent leaf spot intensity varied from 7.99 to 65.32. None of the tested cultivars was found highly resistant to disease. Cultivars *viz*., Eskimo and Gwen showed moderately resistant reaction to *Alternaria dianthi*; while Irene, Gaudina, Amado, Ambrose, Orange viana and White liberty showed moderately susceptible reaction. Bizet showed susceptible reaction; Manuela was highly susceptible to Leaf spot disease.

Table 4. Reactions of carnation cultivars against *Alternaria dianthi* under screen-house conditions

Sr. No.	Cultivars	Per cent leaf spot intensity	Disease reactions
1	Irene	22.22	MS
2	Gaudina	21.33	MS
3	Bizet	40.88	S
4	Eskimo	7.99	MR
5	Amado	21.77	MS
6	Gwen	9.66	MR
7	Ambrose	12.22	MS
8	Orange viana	19.33	MS
9	White liberty	15.77	MS
10	Manuela	65.32	HS

Where:

Reactions		Per cent leaf spot Intensity
Highly resistant (HR)	:	0
Resistant (R)	:	1
Moderately resistant (MR)	:	1.1-10
Moderately susceptible (MS)	:	10.1-25
Susceptible (S)	:	25.1 - 50
Highly susceptible (HS)	:	> 50

5. DISCUSSION

Carnation (*Dianthus caryophyllus* L.) is one of the important cut flower crops grown throughout the world and occupies prime position because of the attractive colour, shape and good vase life. Carnations are exclusively used for cut flowers, bedding, pots, borders, edging, indoors and rock gardens, and also exploited commercially for extraction of perfumes in France and Netherlands. The flower bears especial qualities such as alexiteric, antispasmodic, cardiotonic, diaphoretic and nervine.

Carnation is affected by number of disease even under protected cultivation, among which Leaf spot caused by *Alternaria dianthi* is one of the commonly encountered diseases rendering accountable quantitative and qualitative losses.

The pathogen *Alternaria dianthi* being air borne is very difficult to control with chemicals alone. Therefore, use of botanicals, biological agents and resistant varieties would be reliable and economical for management of the leaf spot disease and successful cultivation of carnation. Considering these facts, present studies on leaf spot disease of carnation were undertaken, during 2015 - 2017 at Plant Pathology Section, College of Agriculture, Pune.

5.1 Symptomatology

At an early stage of disease development, small irregular to circular dark brown spots were observed on lower leaves. After few week of the inoculation concentric rings were formed as a result of irregular growth pattern by the organism in the leaf tissue. These spots tend to be smaller than one centimetre, but especially larger spots were observed on the stem. On expansion the lesions merge and result in blighting of leaves. The infections were generally noticed on lower leaves just above the ground level and

gradually progress upwards. Infection of *A. dianthi* leads to yellowing and blighting of infected plants.

These typical carnation leaf spot symptoms observed in the present study are similar to those described by Seung-Hun *et al.*, (1989) and Sharma (1994). Both scientists reported that *Alternaria dianthi* infection begin as small circular or ovular spots on leaves and stem.

5.2 Isolation and pathogenicity test

The pathogen associated with carnation leaf spot disease was successfully isolated on potato dextrose agar medium, which initially light grey growth and later turned blackish.

The pathogenicity of the isolated pathogen attempted was carried out under screen-house condition on the susceptible variety Manuela by leaf inoculation method conclusively revealed *A. dianthi* as pathogenic to carnation seedlings, at early stage of disease development, small irregular to circular dark brown spot were observed on lower leaves. These circular spots can grow to infect entire plants resulting in wilting or death.

Rodriguez and Juan (1980) and Seung-Hun *et al.* (1989) both scientists also reported that the *Altrnaria dianthi* has been cultured on simple media such as potato dextrose agar. Hajipour *et al.*, (2014) studied 141 isolates belonging to the genus *Alternaria* were isolated and purified. Their pathogenicity was confirmed based on Koch´s postulates.

5.3 Identification of the Pathogen

Based on Leaf spot disease symptomatology (natural and artificially diseased carnation plants), cultural and morphological characteristics and pathogenicity test etc., the test pathogen was identified and confirmed as

A. dianthi. Similar result was obtained by Seung-Hun *et al.,* (1989) who also described the morphology of *A. dianthi*.

5.4 *In vitro* evaluation of bio-agents

In present study, five bio-agents evaluated *in vitro* were found antagonistic against *A. dianthi*. Of these, *Trichoderma viride* and *T. hamatum* were most effective and at par in inhibiting mycelial growth of the test pathogen. The per cent inhibition was highest in the treatment *T. viride* (73.01) and immediately followed by *T. hamatum* (72.15). The other *Trichoderma* spp. showed its in the range 59.09 to 63.35 per cent.

Apet *et al*., (2014) showed antagonistic effect of *T. viride* and *T. hamatum* against *Alternaria alternata* causing leaf spot of gerbera.

Morshed (1985) noticed that the fungus *T. viride* was effective in checking the growth of *A. tenuis.* Similar result is reported by Amaresh (2000) and Rao (2006) against *A. helianthi.*

5.5 *In vitro* evaluation of botanicals

In present *in vitro* study, Clove extract (10%) was found to be most effective followed by Neem leaf extract (10%) and Garlic clove extract (10%) in respect of higher per cent inhibition of mycelial growth of the *A. dianthi*. The per cent inhibition was highest showed by clove extract (91.58%), which is superior over all treatments. The rest of treatments ranged between 34.10 to 56.07 per cent.

Suwitchayanon and Kunasakdakul (2009) reported clove extract most effective against *A. brassicicola*.

Sharma *et al.,* (2007) reported that the neem leaf extract showed high efficacy to inhibit the radial growth of *A. solani*. Similar result has also been

found by Harde and Suryawanshi (2014) against *Alternaria brassicae* (leaf spot of mustard)

Datar (1996) showed the inhibitory effect of garlic clove extract (@ 10 %) on the mycelial growth of *A. tenuis* causing leaf spot of brinjal. Similar result is also found by Kansara and Sabalparae (2015) against *Alternaria alternata* causing leaf spot of niger.

5.6 *In vivo* evaluation of botanicals and bio-agents

Most effective two bioagents *T. viride* and *T. hamatum* and three botanicals Neem leaf extract, Garlic clove extract and clove extract were integrated for management of carnation leaf spot disease, under screen-house conditions. Minimum per cent leaf spot intensity was recorded with clove extract (10%), followed by Neem leaf extract (10%), Garlic clove extract (10%), *T. viride* (0.5 %) and *T. hamatum* (0.5 %). In same order of merit these treatments were also found to control/ reduce carnation leaf spot intensity, over untreated control.

The highest per cent disease control was recorded in Clove extract (90.01%), followed by Neem leaf extract (65.90%), Garlic clove extract (62.73%) *Trichoderma viride* (52.53%) and *T. hamatum* (44.99%).

These results obtained on effectiveness of Clove extract, Neem leaf extract and Garlic clove extract against *Alternaria* spp. are in confirmity with earlier reports by Suwitchayanon and Kunasakdakul (2009), Dubey (2001), Kavita and Dalbeer (2013), Rao (2006) and Sobhy *et al.,* (2014).

As regards to bio-agents efficacy, similar results were documented by Brahmane *et al.,* (2015)

5.7 Varietal Screening and reactions

Of the ten cultivars of carnation evaluated under screen-house conditions, varieties Eskimo and Gwen exhibited moderately resistant reaction to *Alternaria dianthi*. Thus, moderately leaf spot resistant cultivar of carnation, after further confirmation could be brought under commercial cultivation.

6. SUMMARY AND CONCLUSION

Carnation (*Dianthus caryophyllus L.*) is one of the important cut flower crops of the world. Many biotic and abiotic factors limit its production. Among the biotic factors fungal disease are major constraints in profitable production of carnation. Carnation leaf spot caused by *A. dianthi* is exerting a major threat to carnation cultivation under polyhouse conditions.

Hence, present studies on leaf spot of carnation were carried out at the Plant Pathology Section, College of Agriculture, Pune -05 with the following objectives.

1. To prove Koch`s postulate.

2. To evaluate bio-agents & botanicals against leaf spot pathogen *in-vitro.*

3. To evaluate effective bio-agents & botanicals against leaf spot *in-vivo.*

4. To screen available genotypes of carnation against leaf spot

Leaf spot affected carnation samples were collected from National Agricultural Research Project, Ganeshkhind, Pune - 411 007.

The pathogenicity test of isolated was carried out under screen-house condition on the susceptible cultivar Manuela. After the inoculation of isolates, at early stage of disease development, small irregular to circular dark brown spots were observed on lower leaves. After few week of the inoculation concentric rings were formed as a result of irregular growth pattern by the organism in the leaf tissue. The fungus reisolated from leaves inoculated with respect and the pathogenicity was proved successfully for isolates.

On PDA plates, the test pathogen produced septate, profusely branched mycelium with conidia. The size of conidia 42.26 µm in length and 4.29 µm in width. They were light olivaceous to dark brown in colour, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal and 2-10 transverse septa. Based on symptomatology, cultural and morphological characters and pathogenicity test, the pathogen was identified as *Alternaria dianthi*.

Under *in vitro* evaluation, bio-agents *viz., T. viride* and *T. hamatum* and botanicals Neem leaf extract (10%), Garlic clove extract (10%) and clove extract (10%) were found most effective with maximum mycelial growth inhibition of *A. dianthi*.

Also under screen-house evaluation, (*in vivo*), botanicals clove extract (10%) followed by Neem leaf extract (10%), Garlic clove extract (10%) and bio-agents *T. viride* (0.5%) and *T. hamatum* (0.5%), applied as foliar spray were most effective with significant control/reduction of leaf spot disease intensity over untreated control.

The carnation cultivars Eskimo and Gwen were moderately resistant to *A. dianthi.*

The overall result of the different studies indicated that botanical clove extract (10%) most effective for controlling *Alternaria* leaf spot of carnation.

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8. VITA

MISS. PALLAVI DHANAJI GHADAGE

A candidate for the Degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

2017

Title of the Thesis : "STUDIES ON LEAF SPOT OF CARNATION"

Major Field : Plant Pathology

Biographical information:

Personal : Born on 20th July, 1993 at Shindewadi,Tal - Malshiras, Dist - Solapur (Maharastra) Pin-413109. Daughter of Mrs. Pushpa Dhanaji Ghadage and Mr. Dhanaji Ramchandra Ghadage.

Educational :

- Passed S.S.C. examination with First class with Distinction in 2009 from Hanuman Vidyalaya and Junior college, Shindewadi, a/p– Shindewadi, Tal - Malshiras, Dist –Solapur (Maharastra)
- Passed HSC examination with first class with in Distinction 2011 from Malojiraje Sheti Vidyalaya, Phaltan, Tal - Phaltan, Dist –Satara (Maharastra)

- Awarded Bachelor of Science (Agriculture) degree with first Class from College of Agriculture, Pune – 411 005 under Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharastra) in 2015.

Permanent Address **:** A/p - Shindewadi,
Tal - Malshiras,
Dist - Solapur
State - Maharashtra (India).
Pin - 413109

Email **:** pallavighadge96@gmail.com
Contact **:** Mobile - 9657572548

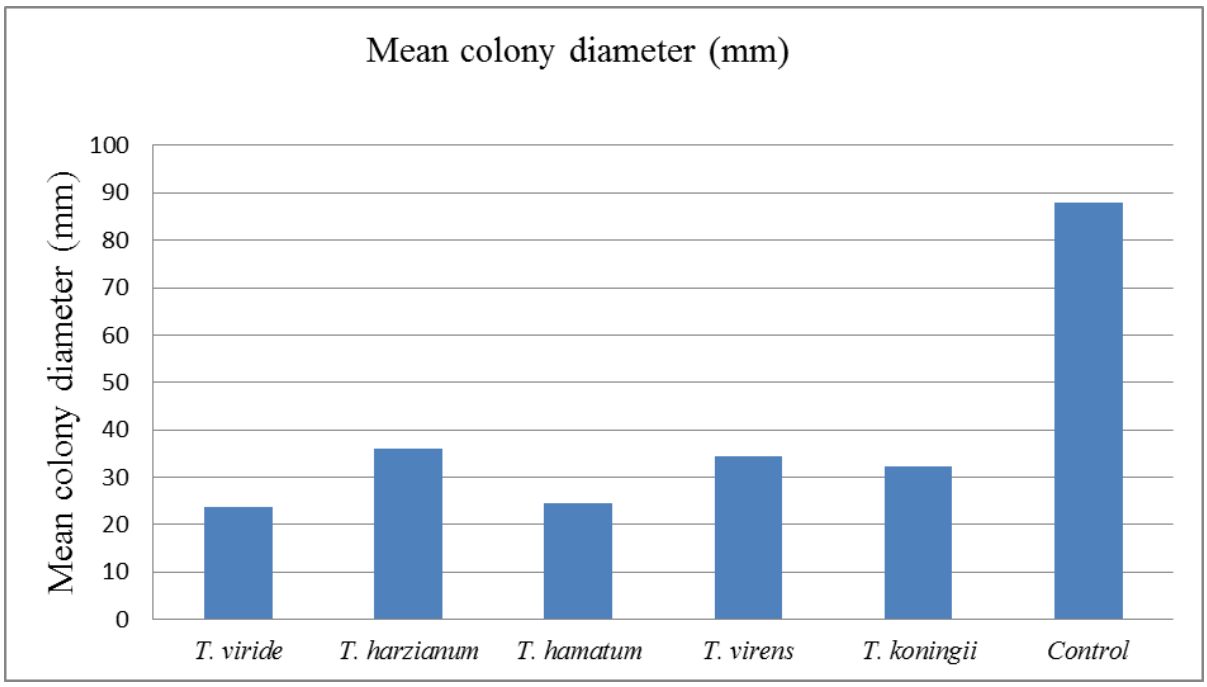

Fig. 1 *In vitro* effect of bio-agents on mycelia growth of *Alternaria dianthi*

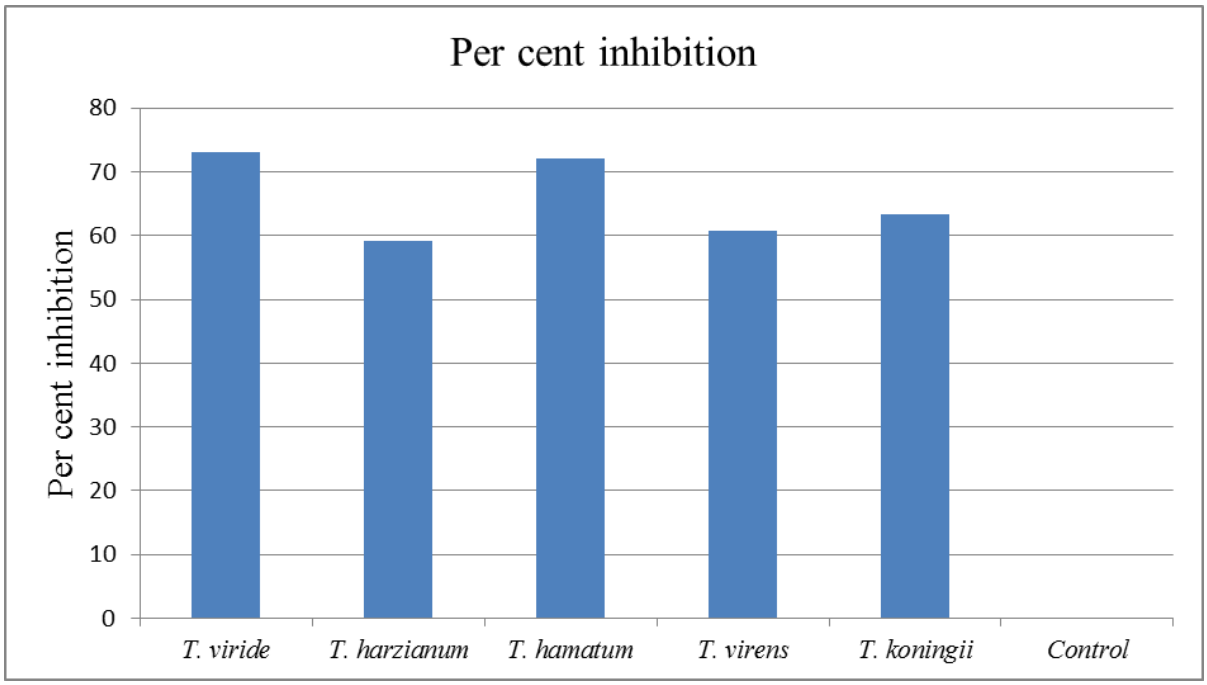

Fig. 2 Per cent mycelia growth inhibition of *Alternaria dianthi* by bio-agents

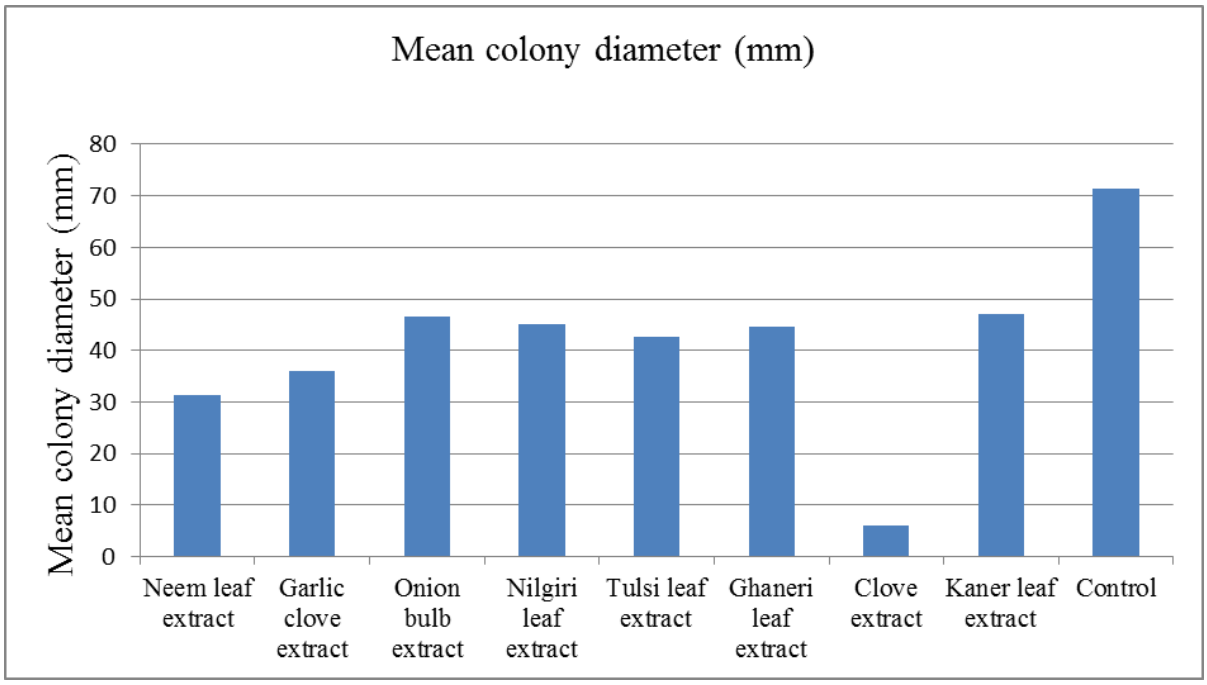

Fig. 3 *In vitro* effect of botanicals on mycelia growth of *Alternaria dianthi*

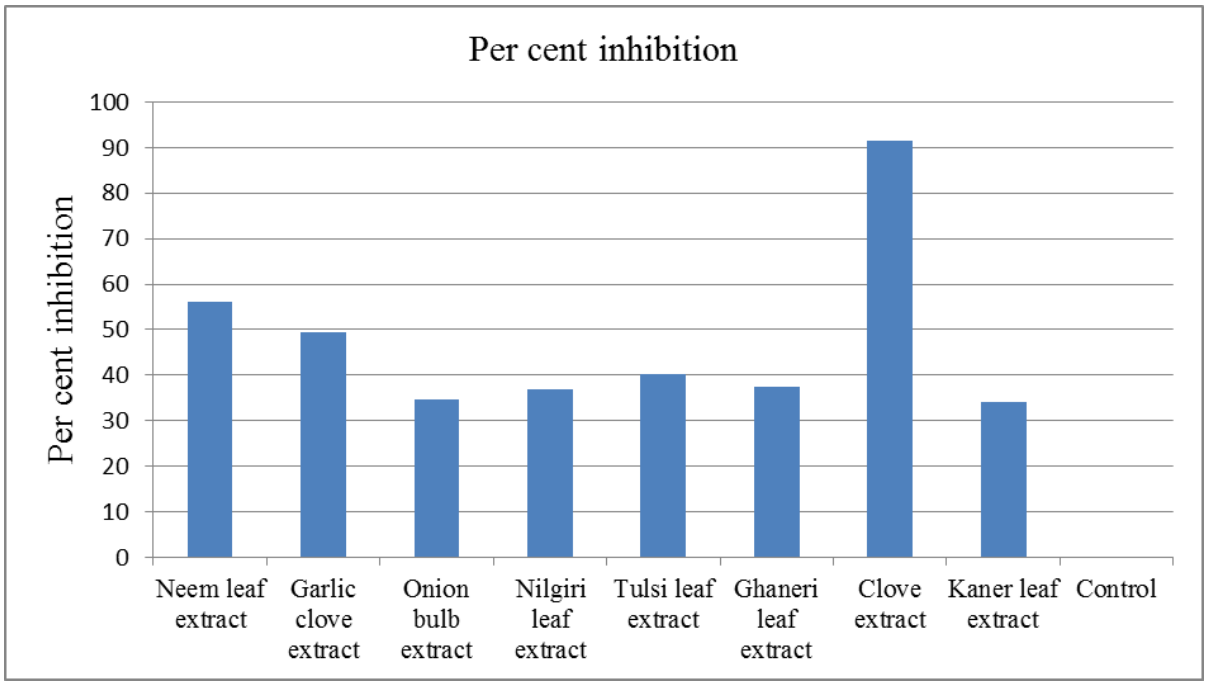

Fig. 4 Per cent mycelia growth inhibition of *Alternaria dianthi* by botanicals

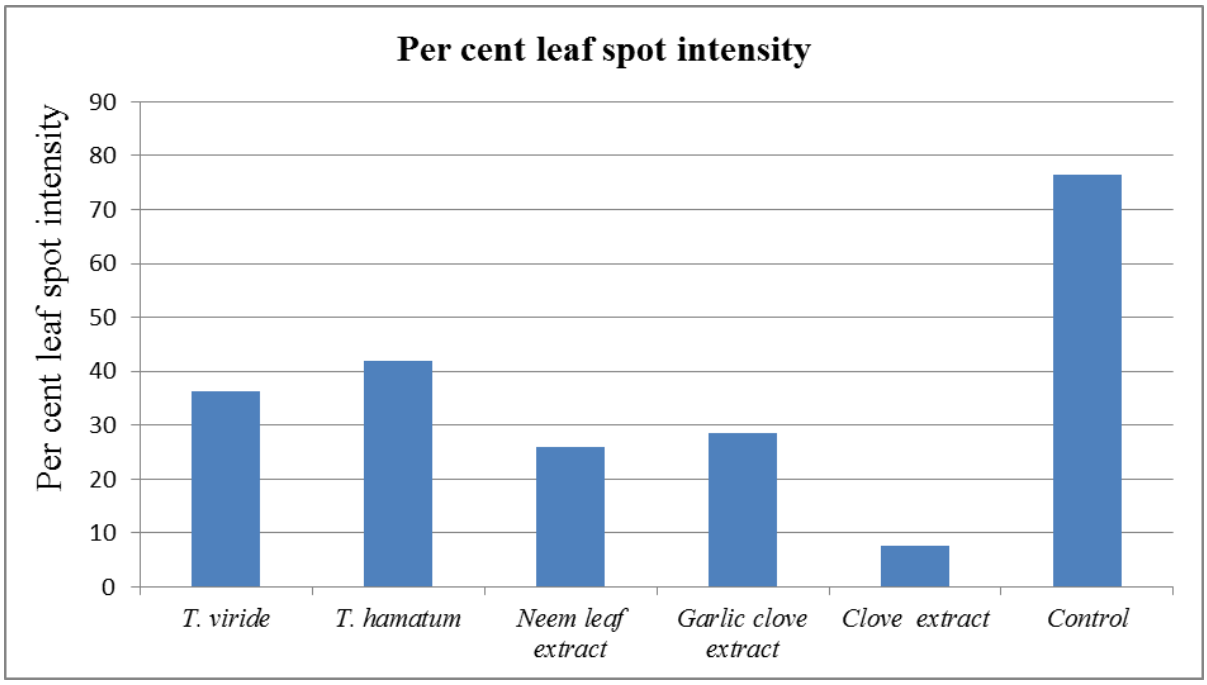

Fig. 5 *In vivo* efficacy of bio-agents and botanicals against *Alternaria dianthi*, causing leaf spot disease of carnation

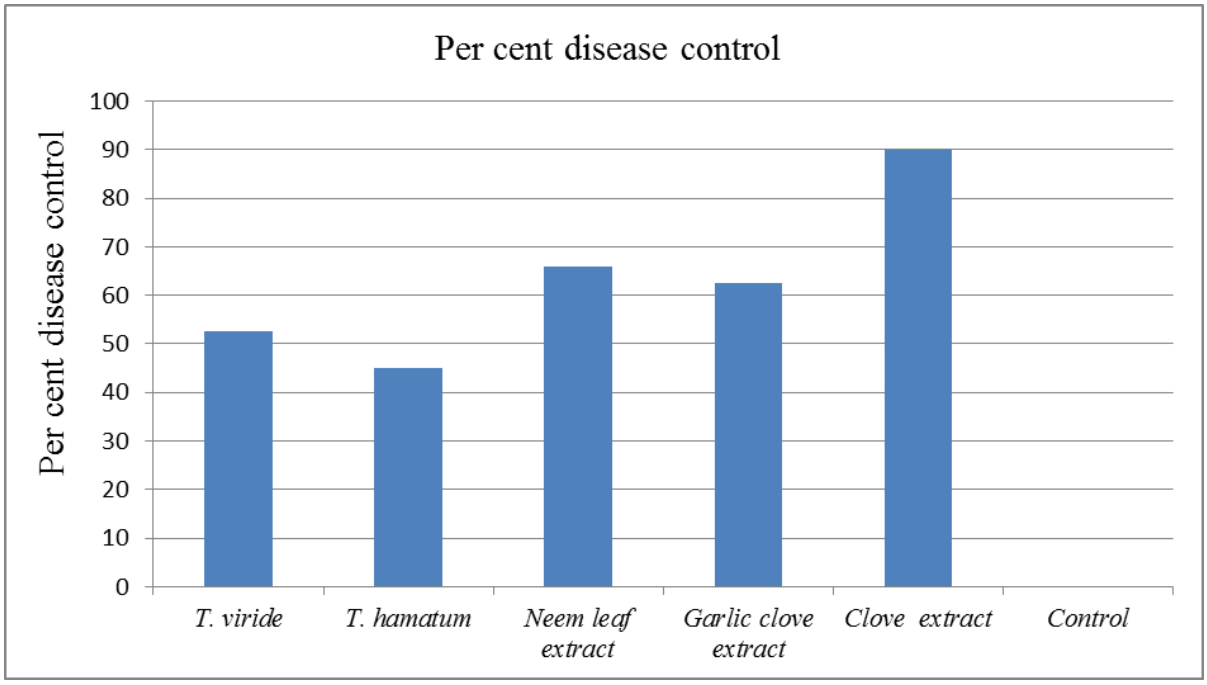

Fig. 6 *In vivo* efficacy of bio-agents and botanicals in control of carnation leaf spot caused by *Alternaria dianthi*

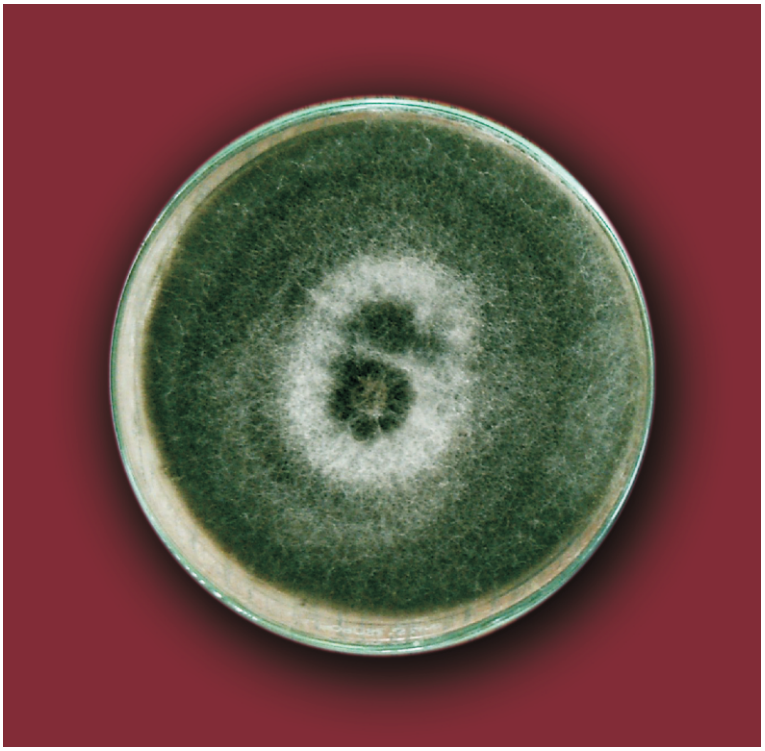

Plate 1. Pure culture of *Alternaria dianthi* on PDA

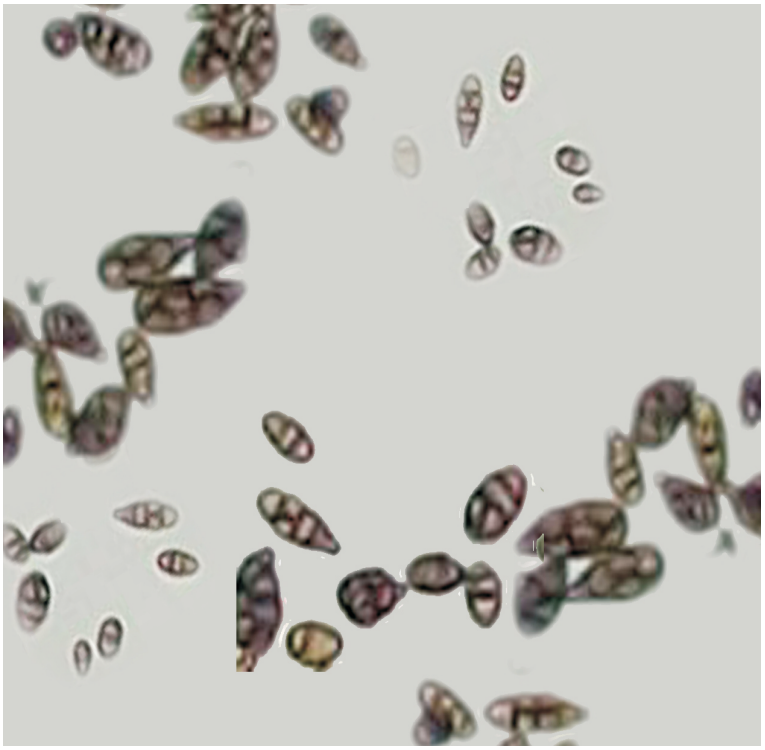

Plate 2. Photograph of conidia of *Alternaria dianthi*

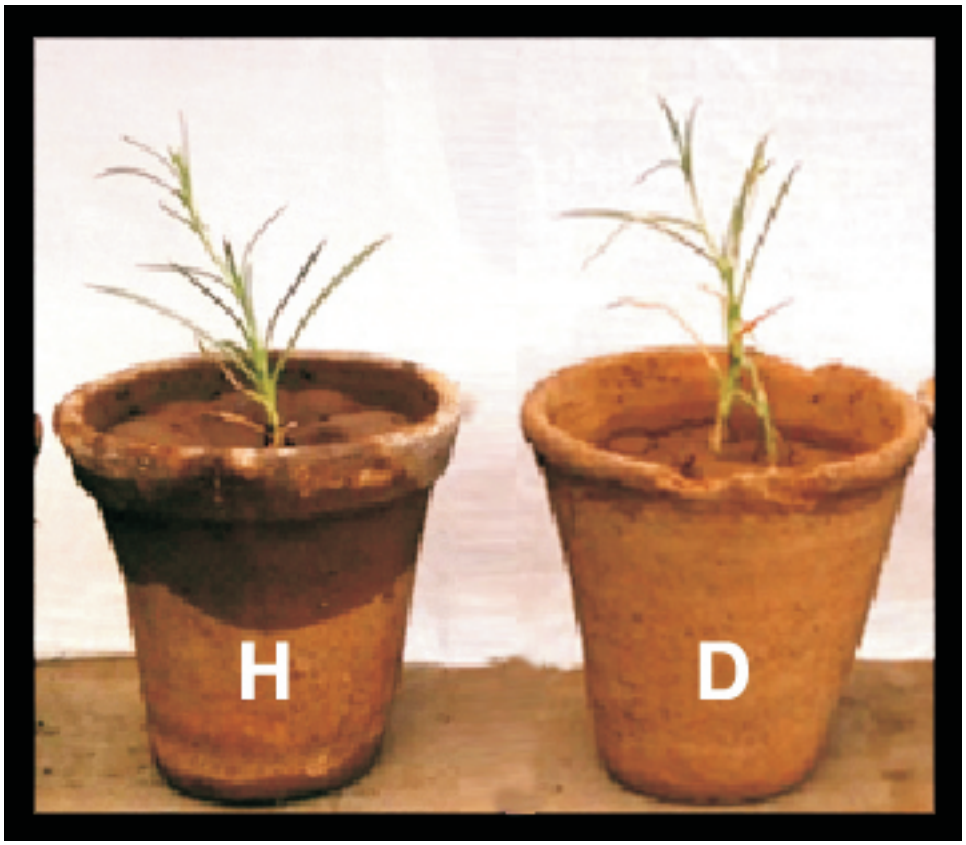

Plate 3. Pathogenicity of *Alternaria dianthi*

H : Healthy D : Diseased

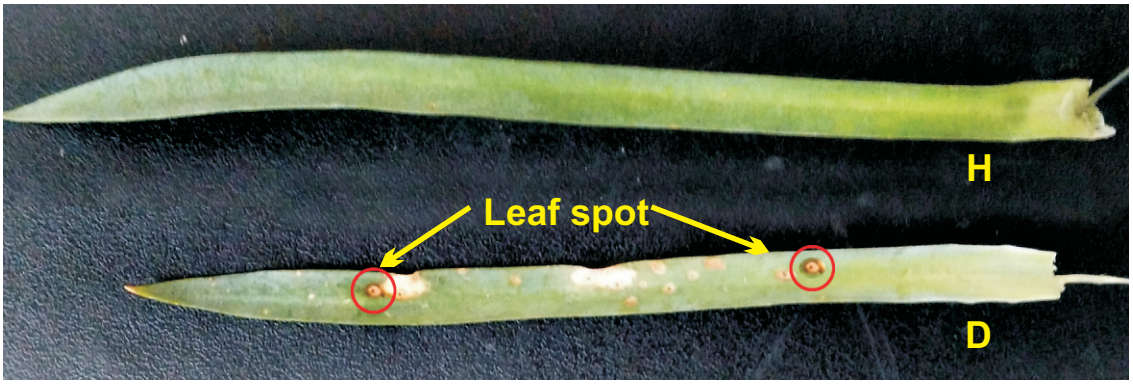

Plate 4. H : Healthy sample

D : Diseased sample

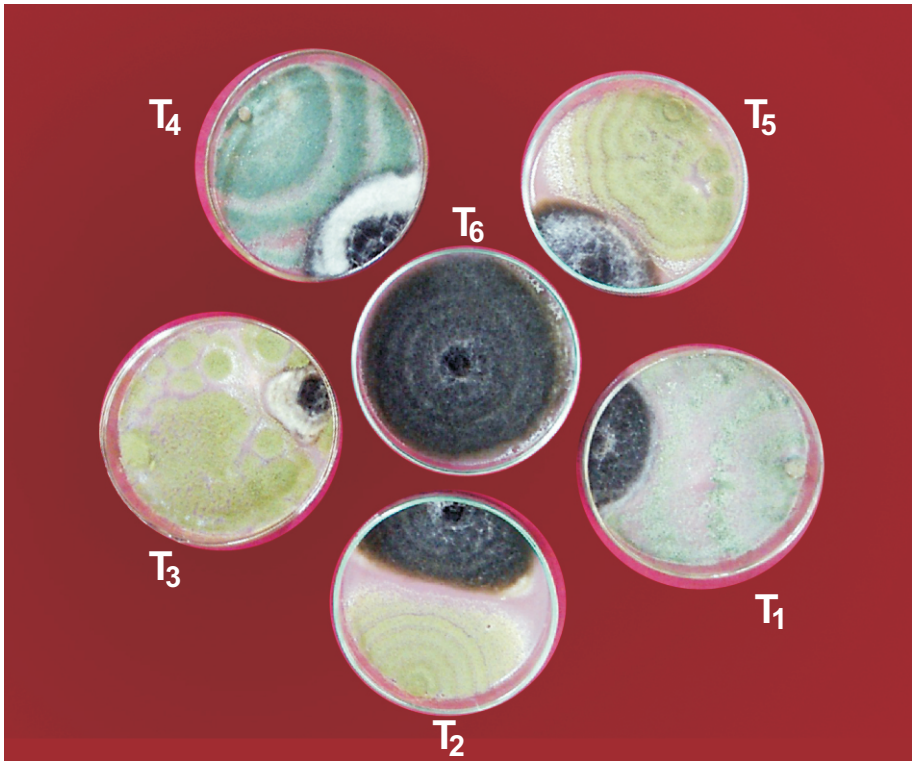

Plate 5. ***In vitro*** **evaluation of bio-agent against** ***Alternaria dianthi***

T_1 : ***Trichoderma viride***

T_2 : ***T. harzianum***

T_3 : ***T. hamatum***

T_4 : ***T. virens***

T_5 : ***T. koningii***

T_6 : **Control (untreated)**

Plate 7. *In vivo* evaluation of effective bio-agents against and botanicals against *Alternaria dianthi*

T_1 : *Trichoderma viride (0.5%)*
T_2 : *T. hamatum (0.5%)*
T_3 : *Neem leaf extract (10%)*
T_4 : *Garlic clove extract (10%)*
T_5 : Clove extract (10%)
T_6 : Control (untreated)

Plate 8. Screening of carnation cultivars against *Alternaria dianthi*

1. Irene
2. Gaudina
3. Bizet
4. Eskimo
5. Amado
6. Gwen
7. Ambrose
8. Orange viana
9. White liberty
10. Manuela

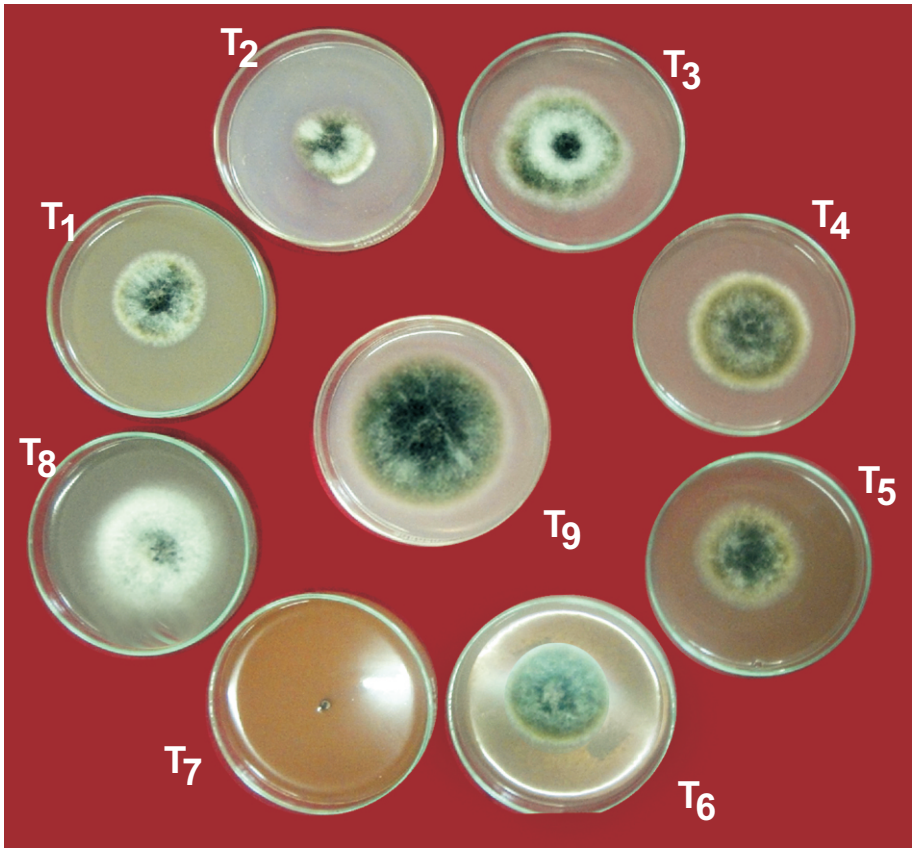

Plate 6. ***In vitro*** **evaluation of botanicals against** ***Alternaria dianthi***

T_1 : Neem leaf extract-10%
T_2 : Garlic clove extract-10%
T_3 : Onion bulb extract-10%
T_4 : Nilgiri leaf extract-10%
T_5 : Tulsi leaf extract-10%
T_6 : Ghaneri leaf extract-10%
T_7 : Clove extract-10%
T_8 : Kaner leaf extract-10%
T_9 : Control