

**MANAGEMENT OF ALTERNARIA LEAF SPOT
OF SOYBEAN**

THESIS

**Submitted to
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
in partial fulfilment of the requirements
for the Degree of**

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

By

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DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of Thesis entitled “**MANAGEMENT OF ALTERNARIA LEAF SPOT OF SOYBEAN**” or part thereof has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis or publication of University or Scientific Organization. The sources of material used and all assistance received during the course of investigation have been duly acknowledged.

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CERTIFICATE

This is to certify that the thesis entitled “**MANAGEMENT OF ALTERNARIA LEAF SPOT OF SOYBEAN**” submitted in partial fulfillment of the requirements for the degree of “**Master of science in Agriculture (Plant Pathology)**” of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafied research work carried out by **ZADE SAMIR BABARAO** under my guidance and supervision.

The subject of thesis has been approved by the student’s Advisory Committee.

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(C) Abbreviations

@	-	At the rate
%	-	Per cent
&	-	and
/	-	Per
°C	-	Degree celcius
CRD	-	Completely Randomized Design
C.D.	-	Critical difference
cm	-	Centimetre
DAG	-	Days after germination
DAI	-	Days after incubation
DAS	-	Days after sowing
Dr. PDKV	-	Dr. Panjabrao Deshmukh Krishi Vidyapeeth
EC	-	Emulsifiable concentrate
e.g.	-	Exempli gratia (For example)
<i>et. al.</i>	-	Et alia (and others)
etc.	-	Et cetra
Fig.	-	Figure
G	-	Gram
H	-	Hactor
i.e.	-	That is
J.-	-	Journal
ml	-	milliliter
mm	-	millimeter
PDI	-	Per cent Disease Incidence
PDI	-	Per cent Disease Intensity
Pf	-	<i>Pseudomonas fluorescens</i>
SD	-	<i>Seed dresser</i>
SC	-	<i>Soluble concentrate</i>
PDA	-	<i>Potato Dextrose Agar</i>
SE (m) +	-	Standard error of mean
Tv	-	<i>Trichoderma viride</i>
<i>viz.,</i>	-	Videlicet (Namely)

(E) THESIS ABSTRACT

a) Title of the thesis : MANAGEMENT OF ALTERNARIA LEAF SPOT OF SOYBEAN

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ABSTRACT

Soybean (*Glycine max* (L.) Merrill) is one of the most important oilseed crop and major growing in Vidarbha region of Maharashtra. It contains 40 percent protein and 20 percent oil. Alternaria leaf spot caused by *Alternaria spp.* is an economical important disease in now days.

The roving survey was conucted in Vidarbha region of Maharashtra in order to know the per cent disease incidence of Alternaria

leaf spot of soybean and results revealed that maximum mean per cent disease incidence (41.66%) was observed in Walgoan village of Amravati district followed by in Mozari (40.19%) Tahsil Tiwsa district Amravati. Whereas minimum percent disease incidence (12.50%) was noticed in village Tuljapur Taluka Seloo district Wardha followed by Jambhrun Parande village in Washim district (13.93%). Among the seven districts surveyed, maximum occurrence of *Alternaria alternata* was observed in Amravati district (90%) followed by Akola (89%), Nagpur (87.5%) and Buldhana district (86.66%). Minimum occurrence of *Alternaria alternata* was observed in Wardha district (83.33%). In comparison to *A. alternata* least occurrence of *Alternaria tenuissima* observed during the course of investigation.

Pathogenicity was proved on soybean plants which are grown on pot conditions which show the symptoms on lower leaves.

Different fungicides were evaluated *in-vitro* by using poison food technique. Hunderd per cent growth inhibition of *Alternaria alternata* was recorded in Hexaconazole, Propiconazole, Propineb and Cymoxonil + Mancozeb treatment. Among the botanicals maximum inhibition was recorded in Garlic clove extract (87.50%).

All the bio-agents tested showed clear significant effect compared with were control. Antagonist *Trichoderma harzianum* and *Trichoderma asperellum* gave the best effect against *Alternaria alternata* forming maximum percent mycelial inhibition i.e. 79.65% and 76.55 %.

Various genotypes were screened against *Alternaria* leaf spot of soybean for the identification of resistant source in available lines. Twelve genotype namely (AMS-2014-C, JS-20-87, AMS-475, AMS-2014-B, NRC-117, SL-20-74, JS-9305, DSB-28-3, RVS-2010-1, AMS-MB-5-19, AMS-32 and AMS-358) exhibited Highly Resistant (HR) reaction. While 14 genotype shown Moderately Resistant (MR) reaction and 4 genotype *viz.* MACS-1460, VLS-58, JS-20-94 and AMS-243 exhibited Moderately Susceptible (MS) reaction. Rest of genotype including checks *viz.* JS-72-44, JS-335 and JS-75-46 shown Susceptible (S) reaction.

CHAPTER I

INTRODUCTION

1.1 Background of Information

Soybean (*Glycine max* (L.)) is one of the most important an Asiatic oil seed crop. It has a prominent place among modern agricultural commodities, as the world's most important seed legume, which contributes 25% to the global edible oil, about two thirds of the world's protein concentrate for livestock feeding and is valuable ingredient in formulated feeds for poultry and fish. Hence, it is spread throughout Asia as well as whole world. People also called to soybean as "Miracle crop" because it is largest source of vegetable oil (18-20%) and protein (40-42%) and other nutrients like calcium, iron and glycine. In the worldwide, USA, Argentina, Brazil and China are largest scale cultivated countries as well as India also larger producer of soybean crop. Among the major soybean growing countries, India ranks fourth in terms of area under soybean and fifth in terms of production (Anonymous, 2018).

In Vidarbha region since 2003-04 onwards soybean emerged as the major *Kharif* crop by replacing sorghum totally and cotton partially. Being a short duration legume crop and as consisting medicinal and soil replenishment properties/qualities befitted in the cropping pattern in Vidarbha especially in crop sequence and intercropping too. Particularly in Vidarbha climatic condition is most suitable for soybean crop therefore only Vidarbha contributed 48.23% area of soybean in Maharashtra (Anonymous, 2017). The area under soybean is consistently increasing every year in Vidarbha region. However due to continuous monocropping, same variety use, seed material exchanges becoming routine and found contributing for increasing severity of foliar diseases of soybean and yield losses year after year. Among the foliar diseases of soybean, *Alternaria* leaf spot is distributed throughout the soybean growing areas of India but it is of special significance in Madhya Pradesh, Maharashtra and Delhi (Gupta and Chauhan, 2005). Members of genus *Alternaria* are cosmopolitan in nature and important fungal pathogen which mostly cause

diseases on aerial parts of many plants worldwide. The members of this genus like *A. alternata*, *A. solani*, *A. porri*, *A. helianthi*, *A. duaci*, *A. carthami*, *A. tenuissima* and *A. macrospora* causes different diseases in their respective hosts (Rostem, 1994). All the aerial parts of the soybean plant are susceptible to *Alternaria spp.* which reduces the quality and quantity of seed yield.

1.2 Importance of Study

The estimates of world soybean area, production and productivity for 2017-18 are 126.64 million ha, 346.31 million tons and 2735 kg/ha and in India soybean grown under area of 10.60 million hectares, production of 8.50 metric tons and average productivity of 802 kg/ha during 2017-18. Madhya Pradesh, Maharashtra and Rajasthan contributes about 92-93% of area and production of soybean in India, however, cultivation of soybean is fast expanding in the states of Telangana, Karnataka and Gujarat (FAO, 2018). In Maharashtra total area under soybean is about 34.5 lakh hectares, production of 2.90 million tons and the average productivity of 841 kg/ha during 2017-18 whereas in Vidarbha soybean crop covered area in 2017 was around 16.63 lakh ha and production was 15.80 lakh metric tons with productivity of 950 kg/ha in 2017 (Anonymous, 2017).

Soybean has already emerged as one of the major *Kharif* crop of the Vidarbha region. Being a short duration legume crop it is an ideal for various intercropping as well as sequential cropping system. Therefore, area and production of soybean has gradually increased although the average productivity is far behind the productivity of world due to various factors including diseases.

Among the causes attributing to low yield, diseases occupy the prime position. Some of the important fungal foliar diseases of soybean were Rust (*Phakopsora pachyrizi*), Cercospora blight (*Cercospora kikuchii*), Alternaria leaf spot (*Alternaria spp.*), Brown spot (*Septoria glycines*), Myrothecium leaf spot (*Myrothecium roridum*), Phyllosticta leaf spot (*Phyllosticta sojicola*), Target leaf spot (*Corynospora cassicola*) and Colletotrichum blight (*Colletotrichum dematium*). Among these leaf spot

incited by *Alternaria spp.* reported to be the most prevalent and widespread disease. Gupta and Chauhan, (2005) reported 10 to 20% reduction in yield due to *Alternaria* leaf spot as resulted on account of premature defoliation, pod and seed decay. Conn and Tewari (1990) reported that among the different foliar diseases, leaf blight caused by *Alternaria*, is one of the most dominant one that causes average yield loss in the range of 32-57 %.

Due to its necrotrophic behaviour destroys chlorophyll of much larger area of lamina than the actual site of infection and hence drastically reduces the photosynthetic area. In congenial condition spots may appear on stem, petioles and on pods. It is well known that the majority of *Alternaria* species are saprophytic fungi. However, some species may appear as significant parasites of cultivated plants (Thomma, 2003). Soybean is infected by species from this genus, most frequently by *Alternaria alternata* and *A. tenuissima* (Sinclair and Backman, 1989; Baird *et al.*, 2001 and Gupta and Chauhan, 2005). These species most frequently cause pathological changes on seed and on leaf and stem.

Since last few years *Alternaria* leaf spot is considered as minor disease in Vidarbha but now days, leaf spot symptoms on soybean are being observed in severe proportion from various places of Vidarbha region on existing cultivated varieties of soybean that becoming potentially threat for soybean cultivation (Anonymous, 2014). Though much work has been done on epidemiology, host range, influence of environmental factors for growth and sporulation, management of disease with chemical fungicides however very scanty information is available on cause and management of *Alternaria* leaf spot of soybean with bio-agent, plant extract and varietal resistance in reference to Vidarbha region. Hence the present investigation aims to find out the distribution of disease, prevalence of precise species of *Alternaria*, efficiency of bio-agents, botanicals, chemical fungicides and screening of the soybean genotypes.

1.3 Objectives of study

- i. Survey *Alternaria* leaf spot of soybean in Vidarbha region of Maharashtra.
- ii. Screening of soybean genotypes against *Alternaria* leaf spot of soybean.
- iii. To find out the effective botanicals and chemical fungicides against *Alternaria* leaf spot of soybean *in vitro*.

1.4 Hypothesis

Among the foliar fungal diseases leaf spot incited by *Alternaria* spp. causes quantitative and qualitative losses to soybean plants. It is quite different to manage in the field in congenial condition. Use of only chemical fungicides is not sole to solve this problem. Control of this disease is more efficient when integrated measures are adopted.

A sufficiently good level of disease control can be achieved by using resistant/tolerant cultivar, botanicals, bio-agents and chemical fungicides against *Alternaria* leaf spot of soybean.

1.5 Scope and Limitation

Since last decades *Alternaria* leaf spot is being reported in severe proportion and damage caused to photosynthetic area could be considerable. It has become a chief contributing factor in the low production of soybean yield.

Management with ecofriendly resources can help in reducing use of hazardous chemicals and turn with protect the environment and soil. Use of bio-agents and botanicals is a useful component of integrated disease management and sustainable agent. Therefore it is required to test the effect of bio-agents, botanicals and certain chemicals in laboratory condition.

CHAPTER II

REVIEW OF LITERATURE

The fungus *Alternaria spp.* is known to infect a wide range of host plants with a high significance and these attributes have made this fungus economically important pathogen. The present investigation was undertaken entitled "MANAGEMENT OF ALTERNARIA LEAF SPOT OF SOYBEAN". The literature pertaining to research is reviewed and presented in this chapter with following subheads-

2.1 Occurrence and losses caused by *Alternaria* leaf spot

Mishra and Prakash (1975) surveyed the soybean crop for *Alternaria* leaf spot diseases in India and reported that disease is caused by *Alternaria tenuissima*.

Chaugule *et al.* (1980) collected fungal leaf spot disease sample from different parts of Maharashtra and made isolation from affected leaves of soybean and they yielded the species of *Alternaria*.

Shortt *et al.* (1982) reported that *Alternaria tenuissima* incitant of leaf spot in soybean having seed borne nature.

Kunwar *et al.* (1986) showed that mature and immature soybean seeds infected with *Alternaria alternata* were small and shrunken with light to dark brown lesions.

Singh (1987) collected the seed samples of soybean from farmer's field and reported that injured seeds carried more fungal infestation than healthy ones. Fungi associated were *Alternaria alternata*, *Fusarium spp.*, *Cercospora kikuchii*, *Macrophomina phaseolina*, *Colletotrichum dematium* and *Rhizoctonia solani*.

Clear *et al.* (1989) reported seed discoloration by *Alternaria spp.* and *Fusarium spp.* that affect quality and production of soybean crop.

Conn and Tewari (1990) reported that among the different foliar diseases, leaf blight caused by *Alternaria*, is one of the most dominant one that causes average yield loss in the range of 32-57 %.

Khan and Sinclair (1992) reported the incidence of *Alternaria alternata*, *Colletotrichum dematium*, *Myrothecium roridum* as foliar pathogens on soybean.

Bhatnagar and Tiwari (1993) reported that Bacterial pustule, Yellow mosaic disease, Rhizoctonia aerial blight, pod blight, rust, *Myrothecium* leaf spot and *Alternaria* leaf spot are the major disease of soybean crop.

Rahman *et al.* (1995) reported the incidence of *Alternaria* leaf spot (*Alternaria alternata*), anthracnose (*Colletotrichum dematium*), *Cercospora* leaf spot (*Cercospora kikuchii*), pod rot and pod blight (*Fusarium semitectum*, *C. kikuchii* and *C. truncatum*) and Rhizoctonia aerial blight (*R. solani*) was higher in early sown crops compared with late sown.

Gupta and Chauhan (2005) reported 10 to 20% reduction in yield of soybean due to *Alternaria* leaf spot as resulted on account of premature defoliation, pod and seed decay.

Pimentel *et al.* (2006) isolated a total of 297 endophytic fungi from 1728 leaf and stem fragments collected at 20 and 40 days after germination from soybean plants grown in the field and a greenhouse. The fungi belonged to eight groups, six dematiaceous genera (*Alternaria*, *Cladosporium*, *Chaetomium*, *Curvularia*, *Drechslera* and *Scopulariopsis*) and the non-dematiaceous genera *Acremonium*, *Aspergillus*, *Colletotrichum*, *Fusarium*, *Paecilomyces* and *Penicillium* along with some Mycelia sterilia were obtained from greenhouse and field-grown plants.

Alka Kushwaha *et al.* (2010) reported that *Alternaria* leaf blight of pigeonpea causes yield losses up to the tune of 40-50 %.

Jasnic *et al.* (2011) conducted the survey for soybean diseases during 2008 and 2009 and they were done phyto-pathological isolation from soybean plants and seed samples from several localities in Serbia. They obtained total 16 isolates of *Alternaria spp.*, 13 from the seed and 3 from both leaf and stem. They also thoroughly investigated cultural,

morphological, molecular and pathogenic characteristics of respective *Alternaria* isolates.

Mueller *et al.* (2012) observed the incidence of *Colletotrichum gloeosporioides*, *C. dematium*, *Alternaria alternata* and *Myrothecium roridum* as foliar pathogens on soybean.

Sharma *et al.* (2014) reported that 15% losses due to *Alternaria* leaf spot in soybean under natural field condition.

Kumar *et al.* (2015) recorded the highest *Alternaria* leaf spot and Target leaf spot disease in the month of October on soybean.

2.2 Survey for incidence of *Alternaria* leaf spot

Kamthane and Rakh (2013) conducted intensive roving survey to record the incidence of *Alternaria* leaf spot disease of soybean during *Kharif* 2012 in Purna and Parbhani district of Marathwada region. Maximum disease severity index 11.0, 49.5 and 66.0% recorded on cultivar JS 9305, JS 335 and JS 71-5 respectively.

Anonymous (2014) conducted survey and surveillance for soybean disease in Amravati district during *Kharif* 2014 and observed maximum 29.60% incidence of *Alternaria* leaf spot disease in Chikhaldara tahsil followed by Dharni (21.30%) and Chandur railway tahsil (22.40%).

Bhosale *et al.* (2014) conducted the survey in different locations of Marathwada region of Maharashtra during *Kharif* 2014. They obtained *Alternaria alternata* in majorly infected leaf samples.

Kale and Ukesh (2015) conducted survey during *Kharif* 2012 in eight districts of Vidarbha region (Akola, Amravati, Wardha, Washim, Nagpur, Buldhana, Chandrapur and Yavatmal) for soybean disease and they observed that among foliar diseases *Alternaria* leaf spot is predominant in Vidarbha region followed by *Colletotrichum* blight.

Anonymous (2016) made survey for soybean disease in Vidarbha region during *Kharif* 2015 and observed maximum 36.17% incidence of *Alternaria* leaf spot disease in Amravati district followed by Akola (30.85%) and Buldhana (24.50%).

2.3 Pathogenicity and symptoms

The fungal pathogen, *A. alternata* mostly affects the foliar parts of soybean and causing light brown to dark brown, roundish-oval to irregular spots of 1 to 2 mm in diameter in initial stage, while later expanded, often coalesced and produced 'Shot hole' during severe infection (Shinde, 1995)

Wada *et al.* (1996) established the pathogenicity of *Alternaria alternata* by spray inoculation method following Koch's postulate in strawberry and reported the outbreak of *Alternaria* black spot of strawberry in Italy caused by the pathotype of *Alternaria alternata*.

Jasnic *et al.* (2011) proved the pathogenicity of *Alternaria tenuissima* in soybean plants. They observed symptoms on inoculated plants such as small necrotic spot with light grey center and reddish to brown margin.

Kamthane and Rakh (2013) isolate the *Alternaria tenuissima* from infected leaves of soybean and proved the pathogenicity on cultivar JS-335. They recorded brown necrotic spot with concentric rings which coalesce and form large necrotic area.

Mamta *et al.* (2013) established the pathogenicity of *Alternaria alternata* by following Koch's postulate in pigeonpea.

Kale and Ukesh (2015) reported that *Alternaria alternata*, *Myrothecium roridum* and *Colletotrichum dematium* isolated from soybean leaves were highly pathogenic to soybean plant.

2.4 Evaluation of fungicides against *Alternaria spp.*

Hiremath and Sundaresh (1985) reported that mancozeb was superior in controlling of *A. alternata* the causal pathogen of leaf blight of soybean.

Amaresh and Nargund (2002) tested efficacy of mancozeb, chlorothalonil, copper oxychloride, iprodione and carbendazim on sunflower and maximum per cent disease intensity of leaf blight was observed in

untreated control while, chlorothalonil treatment found to have lowest percent disease intensity.

Gorawar *et al.* (2006) reported that propiconazole, penconazole and hexaconazole at all three concentrations (0.025%, 0.05% and 0.1%) showed complete inhibition of growth of the *A. alternata*.

Alka Kushwaha *et al.* (2010) evaluated the nine fungicides against the *Alternaria* leaf blight of pigeonpea under field conditions and they reported that Dithane M-45 was most effective in controlling the disease followed by difolatan and chlorothalonil.

Thaware *et al.* (2010) observed that, propiconazole completely inhibited the growth of the *Alternaria alternata* of cowpea causing leaf blight of cowpea.

Pareek *et al.* (2012) carried out the efficacy of systemic and non-systemic fungicide *viz.*, mancozeb, copper oxychloride, carbendazim + mancozeb, carbendazim, thiophanate methyl by poisoned food technique. Mancozeb was found most effective against *Alternaria alternata* followed by copper oxychloride, and carbendazim + mancozeb. Thiophanate methyl was found least effective against mycelial growth and sporulation.

Maheshwari and Krishna (2013) tested various fungicides against *Alternaria alternata* of mungbean and hexaconazole and mancozeb were found most effective.

Kantwa *et al.* (2014) evaluated different fungicides against *Alternaria alternata* causing leaf blight of groundnut *in vitro* and maximum inhibition of mycelial growth over control was recorded in mancozeb (75.46%) followed by copper- oxychloride (71.95%) and carbendazim (61.05%).

Chaitali (2014) tested seven fungicides against *Alternaria cassiae* causing leaf blight in cowpea at 50, 100, 200, 500 and 1000 ppm concentration and recorded that propiconazole inhibited the growth of fungus even at 50 ppm (42.7%) and at 1000 ppm (86%) followed by hexaconazole (93.3%), azoxystrobin (80%) and mancozeb (75.5%).

Singh *et al.* (2014) tested carbendazim, benomyle and vitavax against occurrences of *Alternaria alternata* in mungbean and found that *Alternaria alternata* was most effectively controlled when seed is treated with vitavax and carbendazim.

Amina and Shamim (2016) tested five fungicides at 100, 200, 300, 400 and 500 ppm against *Alternaria alternata* in chickpea and found that tilt and mancozeb were responsible for complete inhibition of radial growth of *Alternaria alternata* at all concentrations followed by carbendazim (73.33%) at same concentration.

Kalieswari *et al.* (2016) tested seven fungicides *viz.*, carbendazim(0.05%), mancozeb (0.2%) copper oxychloride (0.1%), chlorothalanil (0.2%), fosetyl (0.1%), ridomil MZ (0.05%) and dithane M 45(0.05%) against *A. alternata*. Among these fungicides, the minimum diameter of mycelial growth of *A. alternata* (0.75cm) and maximum percentage of inhibition (91.34%) were recorded in mancozeb (0.2%).

Jakatimath *et al.* (2017) evaluated different fungicides *in-vitro* against *Alternaria alternata* and results revealed that tebuconazole and difenconazole showed 100 per cent mycelia inhibition.

Nidhika Rani *et al.* (2018) observed that propiconazole completely inhibited (100%) the mycelial growth and sporulation of *Alternaria spp.*

2.5 Evaluation of botanicals against *Alternaria spp.*

Shekhawat and Prasad (1971) reported that, out of nine plant extracts tested, five plants extract namely *Allium cepa*, *A. sativum*, *Ocimum sanctum*, *Mentha piperita* and *Beta vulgaris* showed strong inhibitory action against *Alternaria tenuissima* isolates from bean.

Chattupadhyay (1999) stated that bulb extract of *Allium sativum* was the best among the several tested botanicals against *Alternaria alternata*.

Thaware (2010) evaluated aqueous leaf extract against *Alternaria alternata* and observed that garlic clove extract showed

maximum mycelial inhibition (63%) followed by neem (33%), karanj (26.66%) and tulsi (27.77%).

Pareek *et al.* (2012) carried out relative efficacy of five plant extracts *viz.*, garlic clove, NSKE (neem seed kernel extract), ashwagandha, bitter melon and bael in different concentrations by poisoned food technique. Garlic clove extract was found most effective in reducing mycelial growth and sporulation of the *Alternaria alternata* followed by neem seed kernel extract however Ashwagandha floral extract was least effective against *Alternaria alternata*.

Bhosale *et al.* (2014) tested different aqueous leaf extract against *Alternaria alternata* of soybean out of which neem, ginger and eucalyptus were found to be highly inhibitory to mycelia growth of *Alternaria alternata*.

Chaitali (2014) reported that Babool leaf extract most inhibitory (56.3%) to *Alternaria cassiae* followed by nilgiri (46.7%), karanj (41.4%) and neem (38.5%).

Gargi Singh *et al.* (2014) evaluated six methanolic extracts of selected plant leaves for their antifungal activity against *Alternaria alternata* at 5, 10 and 20% concentration. Highest reduction in mycelial growth was achieved by *Oleander* followed by *Parthenium*, *Ocimum*, *Lantana*, *Vernonia* and *Eucalyptus* respectively.

Kantwa *et al.* (2014) tested efficacy of seven plant extracts each at five concentrations 50, 100, 200, 500 and 1000 ppm against *Alternaria alternata* of groundnut. Garlic clove extract was found most effective in inhibiting the mycelial growth (46.60%) followed by leaf extract of neem (43.30%), tulsi (35.85%) and rhizome extract of ginger (34.48%).

Regmi *et al.* (2014) reported that leaf extract of *Jatropha curcas* showed maximum mycelial growth inhibition of *Alternaria alternata* (62.9%) followed by *Datura strumarium* leaf extract (55.6%) and *Azadirachata indica* (51.9%).

Singh *et al.* (2014) found that neem and garlic extract were most effective and also showed the least disease incidence of *Alternaria alternata* in mungbean.

Amina and Shamim (2016) tested the antifungal properties of ethanol extracts of *Azadirachata indica*, *Allium sativum* and *Datura metel* at 5%, 10% and 20% concentration against *Alternaria alternata* in chickpea and found that *Allium sativum* was most efficient inhibitor of the *Alternaria alternata* followed by *Datura metel* and *Azadirachata indica*.

Jakatimath *et al.* (2017) evaluated different botanicals *in-vitro* for the control of *Alternaria alternata* and found that onion and garlic bulb extract inhibited maximum mycelial growth of *Alternaria alternata* both at 5 and 10 per cent concentration.

Nidhika Rani *et al.* (2018) reported that garlic clove extract @ 10% concentrations exhibited the maximum (84.31%) inhibition against *Alternaria alternata* whereas 82.18% inhibition of mycelial growth was observed in case of *Alternaria tenuissima* followed by onion and neem extract.

Yogita Thakur and Zacharia (2018) reported that neem leaf extract @ 10% significantly reduced *Alternaria alternata* mycelial growth.

2.6 Efficacy of bioagents against *Alternaria spp.*

Lal and Upadhyay (2002) used three biocontrol agents namely *Trichoderma viride*, *Trichoderma harzianum* and *Gliocladium virens* against *Alternaria tenuissima* the incitant of leaf blight of pigeonpea *in vitro*. In dual culture, *T. viride* overgrew the colony of *A. tenuissima* within 72 hours of incubation followed by *G. virens* and *T. harzianum*, which took more than 96 hours to completely overgrow the colony of test fungus. The radial growth of *T. viride* was more in mono and dual culture than *G. virens* and *T. harzianum*.

Thaware *et al.* (2010) observed that *Trichoderma harzianum* and *Trichoderma viride* significantly inhibited the mycelial growth of *Alternaria alternata* by 85.88% and 81.88% respectively.

Mumtaz *et al.* (2012) tested *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *Alternaria alternata* in oil seeds crops and found that *Trichoderma viride* was most effective with significant mycelial inhibition (67.6%) followed by *Pseudomonas fluorescens* (66%) and *Bacillus subtilis* (53.3%).

Pareek *et al.* (2012) carried out the relative efficacy of biocontrol agent's viz., *Trichoderma harzianum*, *T. viride* and *Aspergillus niger* by in dual culture plate method and found that *T. harzianum* was found most effective antagonist against *A. alternata* followed by *T. viride* and *A. niger*.

Behairy *et al.* (2014) tested the efficacy of bioagents viz. *Trichoderma album*, *Trichoderma harzianum*, *Bacillus subtilis*, *B. megaterium*, *B. lechniformis* and fungicides Tridex 80% WP under *in vitro*, greenhouse and field condition against *Alternaria* leaf spot of faba bean and they reported that *Bacillus subtilis* and *Trichoderma harzianum* significantly affected the causal pathogen (*A. alternata*) in both the conditions respectively.

Chaitali (2014) tested *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *Alternaria cassiae* in cowpea and found that *Trichoderma harzianum* was most effective with significant mycelial inhibition (83.3%) followed by *Trichoderma viride* (81.4%), *Pseudomonas fluorescens* (66%) and *Bacillus subtilis* (67.8%).

El-Mougy *et al.* (2016) studied the efficacy of biocontrol agents like *Trichoderma harzianum*, *Pseudomonas fluorescence* and *Saccharomyces cerevisiae* in *in-vitro* against *Alternaria tenuissima*. Fungal growth decreased by 70.0, 76.5, and 76.5% by the antagonists *Pseudomonas fluorescence*, *Trichoderma harzianum* and *Saccharomyces cerevisiae* respectively.

Jakatimath *et al.* (2017) evaluated the bioefficacy of fungal and bacterial antagonist against *Alternaria alternata* by dual culture

technique and they revealed that fungal bio agents were better than bacterial bioagents in inhibiting the growth of *Alternaria alternata*.

Nidhika Rani *et al.* (2018) reported that rate of mycoparasitism was faster in *Trichoderma viride* (Pusa isolates) providing 80.36% inhibition of growth of *Alternaria alternata* whereas *Trichoderma viride* (Pusa isolates) provided 74.27% inhibition of growth in case of *Alternaria tenuissima*.

2.7 Screening for disease resistance against *Alternaria* leaf spot

Shehzad *et al.* (2006) conducted preliminary studies on screening of 99 soybean entries against bacterial pustule, anthracnose, fungal leaf spot and Yellow Mosaic Virus under natural field condition and observed disease intensity of varying degrees.

Behairy *et al.* (2014) evaluated 16 different cultivars of faba bean (*Vicia fabae*) in Egypt against aggressive isolate of *A. alternata* under epiphytotic condition and they revealed that Wadi-I cultivar was the most susceptible while Giza 429, Nobarria 2, Sakha 2 and Nobarria 1 cultivar were the most resistant ones.

Mahesha *et al.* (2009) evaluated 204 soybean genotypes under natural and laboratory conditions against major disease and reported several genotypes having multiple resistance source.

Anonymous (2015) tested 40 genotypes under natural field condition against root rot, YMV and *Alternaria* leaf spot and results revealed that 23 genotypes exhibited resistant reaction while rest of genotype shown susceptible reaction.

Ingle *et al.* (2015) assessed the 42 selected elite lines of soybean for resistance to *Alternaria* leaf spot disease under natural field condition and results revealed that 15 genotypes shown absolute resistant reaction, 7 highly resistant reactions, 9 moderately resistant reactions while rest of the genotype exhibited moderately susceptible to susceptible reaction.

Ingle *et al.* (2016) screened 30 advance lines of soybean against *Alternaria* leaf blight, Yellow Mosaic Virus, Bacterial pustules and

Root rot in protected and unprotected conditions under natural field conditions and results revealed that four entries viz NRC 94, JS 20-69, JS 20-71 and KDS 699 were shown resistant high yield (R-HY) reaction to disease complex while NRC-92, KDS 705, KDS 378 and JS-20-41 exhibit susceptible high yield tolerance (S-HY (T)) reaction to disease complex. Rest of the tested advance lines showed susceptible low yield (S-LY) reaction to disease complex.

Anonymous (2016) evaluated sixteen existing cultivar of soybean against *Alternaria* leaf spot diseases and observed disease incidence in the range of 0.0 to 44.15% on respective cultivar and maximum per cent incidence being observed on JS-72-46 (44.15%) followed by JS-75-46 (39.89%), VLS-58 (35.16%), JS 71-05 (34.62%). JS-9305 and Punjab-1 was found *Alternaria* leaf spot disease free.

Sunil Kumar and Pardeep Kumar (2017) evaluated the effect of date of sowing and varieties (namely JS-9305, JS-335, JS-9752 and RKS-18) of soybean on severity of *Alternaria* leaf spot at Medziphema (Nagaland). The crop sown on 15th June exhibited lowest *Alternaria* leaf spot in cv. JS-9752 over the rest of the varieties.

CHAPTER III

MATERIAL AND METHODS

The present investigations on “Management of Alternaria leaf spot of soybean” were conducted during 2017-2018 at the Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The materials used and methods adopted during the present investigation are described in this chapter.

3.1 Materials

Following materials were used for experimentation.

3.1.1 Glasswares

The glassware's used during the course of Research work were of Borosil make. The glassware's viz. Petri plates, Test tubes, Conical flasks of 250 ml, 500 ml and 1000 ml, Funnel, Beakers, Pipettes, Measuring cylinder, Slides, Cover slips and Glass rods were used.

3.1.2 Equipments used

Standard laboratory equipments viz., Autoclave, BOD incubator, Laminar air flow, Research microscope, Stereoscopic Binocular microscope, Refrigerator, Hot air oven, Digital weighing balance, Bunsen burner, Water distillation unit etc. were used.

3.1.3 Soybean genotype seed

The seeds of soybean genotype along with checks were received from AICRP on Soybean scheme, Amravati (MS).

3.1.4 Source of fungicides, bioagents and botanicals

The fungicides viz., as Mancozeb, Carbendazim, Mancozeb + Carbendazim (combi product), Hexaconazole, Propiconazole, Copper oxychloride, Propineb, Thiophanate Methyl, Tebuconazole + Trifloxystrobin (combi product) and Chlorothalonil, Cymoxonil + Mancozeb and bioagents, *Trichoderma asperellum*, *Trichoderma atroviride*, *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescence* and aqueous leaf extract of *Azadirachta indica* (Neem), *Parthenium hysterophorus* (Parthenium),

Lantana camara (Ghaneri), *Lawsonia innermis* (Mehandi), *Datura metal* (Datura), *Allium sativum* (Garlic), *Eucalyptus spp.* (Nilgiri), *Ipomea carnea* (Beshram), *Zingiber officinale* (Ginger), *Curcuma longa* (Turmeric) and *Alium cepa* (Onion) used during studies.

3.1.5 Other materials used

Plastic petriplates, Blotter paper, Non absorbent cotton, Muslin cloth, Polyethylene bags, Cork borer, Inoculation needle, Dissection needle, Forceps, Paper bags, Pencil, Permanent marker, Cello tapes, Whatman filter paper (4 mm), Test tube stand, Tray, Knapsack spray, Potato, Rubber band, Mortar and pestle, etc. were used during Research programme.

3.2 Methods

3.2.1 Survey

A Rowing survey was conducted at Vidarbha region of Maharashtra during the *Kharif* season 2017, to know the disease incidence of Alternaria Leaf Spot of soybean. Under natural field conditions, Amravati, Akola, Buldhana, Nagpur, Wardha, Washim, Yavatmal District were surveyed. Alternaria Leaf spot observed during the crop growth period in respective district. Observations of these diseases were recorded according to the uniform method of disease rating as per the recommendations of (45th) Annual Group Meeting (2015-16) of All India Coordinated Research Project on Soybean.

3.2.2 Sterilization of glassware's and other materials

The cleaned and dried glassware's sterilized in Hot air oven at 180°C for 1 hr. Culture media and distilled water was sterilized in autoclave at 1.05 kg/cm² for 20 minutes. Soil sterilization was done with the help of 10% formalin solution. Soil was mixed with 10% formalin solution and then covered with the polythene for 5 days or soil sterilized in an autoclave at 30 lbs PSI.

3.2.3. Collection of disease sample

Diseased leaves, of soybean were collected during survey, from Akola, Amravati, Wardha, Washim, Nagpur, Buldhana and Yavatmal districts during the period of July to September 2017 and collected diseased samples brought in laboratory to identify the cause.

3.2.4. Preparation of Potato Dextrose Agar medium (PDA)

The Potato Dextrose Agar medium was used as basal medium for *in vitro* studies and for maintenance of pure culture of *Alternaria spp.* in slants.

For mass multiplication of fungi Potato Dextrose broth (PDB) were used.

Composition of PDA medium

Peeled potatoes	- 200 g
Agar-agar	- 20 g
Dextrose	- 20 g
Distilled water	- 1000 ml

200 g peeled potatoes were sliced into pieces and boiled in 500 ml distilled water till properly cooked. The extract was strained through muslin cloth and measured. In the remaining water after dissolving 20 g agar and 20 g dextrose, potato extract was added and the volume was made to one litre. The medium was distributed in flasks and tubes and sterilized in autoclave at 1.04 kg /cm² for 15 min. The slants were used for maintenance of culture and the medium in flask for isolation of fungus.

3.2.5. Isolation of pathogen by tissue isolation method

Isolation of fungal pathogens from the infected soybean leaves used for isolation by tissue method. Infected lesions were selected and centre core of infected tissues separated in pieces of 5 mm, surfaces disinfected with 0.1% HgCl₂ for two minute. These pieces were then placed in the sterilized distilled water and bits after dried on sterilized filter paper and around flame of spirit lamp were placed on sterilized solidified PDA medium in plate. Each plate contained five bits. The plates were

incubated at room temperature ($28\pm 2^{\circ}\text{C}$). All these operations were carried out aseptically. The plates were examined regularly. The fungus colonies growing around the each bit were examined and sub cultured. Based on colony character, morphological characters (types of conidia) and published literature the fungi were identified as *Alternaria alternata* and *A. tenuisiima*. The pure culture was transferred on PDA slants and maintained for further studies.

3.2.6 Per cent occurrence of pathogen

During the survey number of infected leaf samples collected from the fields and each sample was taken in brown paper bag and labeled immediately. The collected samples were further analyzed and used for isolation and detection of pathogen. Total 440 bits (leaf tissue) subjected for isolation and yielded pathogen counted as per cent occurrence by using following formula-

$$\text{Per cent occurrence} = \frac{\text{No. bits yielded pathogen}}{\text{No. of bits used for isolation}} \times 100$$

3.2.7. Purification and maintenance of fungal culture

Culture was purified by following hyphal tip method and culture obtained was maintained on Potato Dextrose Agar (PDA) medium slants by adopting subsequent sub culturing at periodical, regular intervals. Seven days old culture was used for further studies.

3.2.8. Preparation of spore suspension for pathogenicity test

Seven days old growth of the fungi was scrapped from lawn culture of PDA and mixed in distilled sterilized water separately. The bits of medium were removed by sedimentation and by filtering through three folds of muslin cloth and pure spore suspension was obtained.

3.2.9. Pathogenicity test

Seven days old culture of the organism was used for proving the pathogenicity by applying Koch's postulates. For this purpose, seeds of soybean cultivar JS-335 which is susceptible to *Alternaria* leaf spot

were surface sterilized with 0.1 % HgCl₂ and sown in the plastic pots filled with steam sterilized potting mixture of soil: sand: FYM (2:1:1). Healthy growing soybean seedlings were maintained, watered regularly and kept in the shade net green house for further development. Three weeks old healthy seedlings were selected for inoculation. The spore suspension was prepared and filtered through two layers of sterile muslin cloth to remove residual mycelia. Filtrate obtained was suitably diluted with sterile distilled water to get inoculum concentration of 1 x 10⁶ spores per ml. The seedlings firstly injured with carborandom powder then were inoculated with seven days old test fungus. Un-inoculated seedlings of the same age sprayed with sterilized water served as control. After inoculation, the seedlings pots (both inoculated and un-inoculated) were incubated in the green house, where relative humidity (80-90%) and optimum temperature (27±1°C) were maintained for further development of *Alternaria* leaf spot symptoms. Re-isolation was made from inoculated leaves by the isolated fungus which resembled in all respect with the culture used for inoculation.

3.2.10. Re-isolation

Artificially infected leaves of soybean were used for re-isolation of the test pathogens. The cultures obtained were compared for growth with original cultures obtained from naturally diseased specimens of soybean to confirm their identity and pathogenicity.

In present study, higher per cent occurrence *A. alternata* observed in comparison to *A. tenuissima* in infected leaf samples at different locations so that for further studies *A. alternata* pure culture was used.

3.2.11. In-vitro evaluation of fungicides against *Alternaria alternata*

The 11 fungicides (Table 1) were evaluated by employing poison food technique (Nene and Thapliyal, 1993). Potato Dextrose Agar (PDA) medium was prepared, equally distributed measuring 100 ml in 250 ml conical flask and sterilized in autoclave. Requisite quantity of each of the fungicides (as per concentration) was added in sterilized melted (45°C)

PDA separately so as to obtain desired concentration. Flask containing poisoned medium was shaken well to have even and uniform distribution of fungicides. About 20 ml of melted poisoned PDA was poured in each sterilized Petri plate and allow solidifying. These Petri plates were inoculated by test fungus separately. Five mm disc of one week old fungus culture was cut with sterilized cork borer, lifted and transferred aseptically in the centre of Petri plate containing the medium poisoned with test fungicide. The control plates were kept the culture disc grown in same condition on PDA without fungicides. Treated plates were incubated at room temperature ($25 \pm 2^{\circ}\text{C}$) for a period of seven days. Colony diameter was recorded in mm and per cent mycelial growth inhibition was calculated as per Vincent's formula based on the average colony diameter. The data was subjected to statistical analysis wherever necessary.

$$\text{PI} = \frac{\text{C}-\text{T}}{\text{C}} \times 100$$

Where,

PI = Per cent Inhibition

C = Growth of fungi in control (mm)

T = Growth of fungi in treatment (mm)

Table 1. List of fungicides used

SN	Chemical name	Trade name	Company	Conc. (%)
1	Mancozeb 75 % WP	Indofil M-45	Indofil Industries Ltd.	0.2
2	Carbendazim 50 %WP	Starbenz	Swal Co-operation Ltd.	0.1
3	Mancozeb 12%+ Carbendazim 63 % WP	Saaf	UPL India Ltd.	0.2
4	Hexaconazole 5 % EC	Contaf	Tata Rallis India Ltd.	0.1
5	Propiconazole 250 % EC	Tilt	Syngenta Pvt. Ltd	0.1
6	Copper Oxychloride 50 % WP	Blitox 50	Tata Rallis India Ltd.	0.3
7	Propineb 70 % WP	Antracol	Byer Ltd.	0.2
8	Thiophanate Methyl 70 % WP	Roko	Nippon soda Co. Ltd.	0.2
9	Tebuconazole 50% + Trifloxystrobin 25% WG	Nativo	Byer Ltd.	0.1
10	Chlorothalonil 75 % WP	Kavach	Syngenta Pvt. Ltd.	0.2
11	Cymoxanil 8%+Mancozeb 64 % WP	Custom	Bharat Insectides Ltd.	0.1

3.2.12. *In-vitro* evaluation of botanicals against *Alternaria alternata*

The poisoned food technique (Nene and Thapliyal, 1993) as desired in 3.2.6 was employed to evaluate the efficacy of various botanicals against *Alternaria alternata*.

Preparation of aqueous leaf extract of Botanicals

The plant leaves extract were prepared by adopting aqueous extracting method. The standard aqueous leaf extract of the selected botanicals (Table 2) was obtained by grinding the washed plant leaves (100 g) in mortal and pestle in presence of equal amount of sterilized distilled water (100 ml). Prepared leaves extract were filtered through folds of muslin cloth, make up the volume and treated as 100% extract. Colony diameter was recorded in mm and per cent of mycelial inhibition was calculated as per formula given below based on the average of colony diameter. The data of mycelial growth was also subjected to statistical analysis and conclusion was drawn.

Table 2. List of botanicals used

SN	Common name	Botanical name	Part used	Concentration (%)
1	Neem	<i>Azadirachta indica</i>	Leaves	10
2	Cogress grass	<i>Parthenium hysterophorus</i>	Leaves	10
3	Ghaneri	<i>Lantana camara</i>	Leaves	10
4	Mehandi	<i>Lawsonia innermis</i>	Leaves	10
5	Datura	<i>Datura metal</i>	Leaves	10
6	Garlic	<i>Allium sativum</i>	Clove	10
7	Nilgiri	<i>Eucalyptus spp.</i>	Leaves	10
8	Beshram	<i>Ipomea carnea</i>	Leaves	10
9	Ginger	<i>Zingiber officinale</i>	Rhizome	10
10	Turmeric	<i>Curcuma longa</i>	Rhizomes	10
11	Onion	<i>Alium cepa</i>	Bulb	10

3.2.13. *In-vitro* evaluation of bioagents against *Alternaria alternata* by dual culture technique

The lawn culture of test fungi and bio-agents i.e. *Trichoderma asperellum*, *Trichoderma atroviride*, *Trichoderma harzianum* were prepared. Autoclaved melted Potato Dextrose Agar was poured in Petri plates and allowed to solidify for obtaining leveled surface. The plates were inoculated with the culture of test fungi and bioagents after solidification of media and then plates were incubated at room temperature for seven days.

Bacterial bioagents, *Bacillus subtilis* and *Pseudomonas fluorescence* were prepared by inoculating a loopful culture in sterilized conical flask containing hundred ml of nutrient broth. Broth culture was incubated at room temperature for three days. Five mm disc of one week old test fungus and bio-agent lawn culture was cut with the help of cork borer lifted and transferred in Petri plates, containing autoclaved solidified PDA medium. In each Petri plates, four discs of bioagents were inoculated at four peripheral points of the plates and the test fungi was placed in centre of Petri plates. In case of *Pseudomonas fluorescence* and *Bacillus subtilis*, a three days old culture was streaked around the disc of test fungus. The test fungi grown in same condition on Potato Dextrose Agar without bioagents served as control. All these plates were incubated at room temperature for seven days. After an expiry of seven days incubation period the mycelial inhibition was calculated as per formula mentioned in the poisoned food method.

3.2.14. Screening of genotypes

Under natural field conditions, 33 genotypes along with checks were evaluated at the field of Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola in a non-replicated manner with 3 rows of 3 meter length.

1. Location : Plant Pathology field, Dr. P.D.K.V. AKOLA
2. Soil type : Vertisol.
3. Design : Non replicated trials
4. Plot size : 3 rows of 3 m length
5. Season : Kharif
6. Entries : 27 + 6 ©=33
7. Date of sowing : 4 July 2017
8. Method of sowing : Dibbling
9. Fertilizer dose : 30 kg N, 75 kg P, 30 kg K/ha.

Plant protection measures: Nil

The observation on leaf spot infection were recorded at 30 and 65 days after sowing by selecting three leaves each from top, middle and lower portion of the plant. The observations were recorded on the basis of 0-9 scale (Mayee and Datar, 1986).

Disease rating scale

Grade	Description
0	No infection
1	1 % leaf area covered with lesions/spots
3	1.1 – 10 % leaf area covered by lesions/spots
5	10.1 – 25 % of the leaf area covered by lesions/spots; no defoliation; little damage.
7	25.1 – 50 % leaf area covered by lesions/spots; some leaves drop; damage conspicuous
9	More than 50 % area covered by lesions/spots, very common on all plants, defoliation common;

The above rating scales or grades are utilized for the calculation of PDI using the following formula -

The average intensity of each plot was worked out by using formula

RI	AMS 2014-C	TAMS- 38 ©	AMS- 105	AMS- MB-5-18	JS- 20-87	JS-20- 69 ©	AMS- 475	JS-20- 29 ©	PS- 1569	AMS- 2014-B	JS-72- 44 ©
RII	NRC- 117	SL-20- 74	AMS- 56	JS-9305 ©	AMS- 1001	MACS- 1460	AMS- 1004	DSB- 28-3	KDS- 869	AMS- 102	VLS-58
RIII	RVS- 2010-1	JS-335 ©	AMS- 38-24	AMS- MB-5-19	JS- 75-46 ©	AMS- 77	JS-20- 94	AMS- 243	AMS- 32	AMS- 353	AMS- 358

Fig. 1 Plan of layout

$$\text{Percent Disease Intensity (PDI)} = \frac{\sum \text{ of all numerical ratings}}{\text{Total number of leaves examined} \times \text{maximum ratings}} \times 100$$

On the basis of PDI, the entry/variety can be classified as follows:

	PDI		Resistant category
1st =	0	=	Absolutely Resistant (AR)
2nd =	0.01 – 11.11	=	Highly Resistant (HR)
3rd =	12.22 – 33.33	=	Moderately Resistant (MR)
4th =	34.44 – 55.55	=	Moderately Susceptible (MS)
5th =	56.66 – 77.77	=	Susceptible (S)
6th =	78.88 – 100.00	=	Highly Susceptible (HS)

3.2.15 Grain yield (kg/plot)

The grain yield kg/plot was recorded after threshing, cleaning and drying up to moisture 9% and then weighted the yield with the help of electronic balance.

3.2.16 Statistical analysis

The data of each character were subjected to statistical test by applying of variance technique (Panse and Sukhatme, 1985).

CHAPTER IV

RESULTS AND DISCUSSION

The present studies on “MANAGEMENT OF ALTERNARIA LEAF SPOT OF SOYBEAN” was conducted at Department of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola during 2017-2018 and observations recorded on different parameters during the studies are presented below-

4.1 Roving survey of Alternaria leaf spot disease in Vidarbha region

An intensive roving survey for Alternaria leaf spot disease was conducted during *Kharif* 2017 to know the incidence of Alternaria leaf spot in farmer fields of Vidarbha region.

The disease incidence in respective places presented in Table 3. Maximum mean per cent disease incidence (41.66%) was observed in Walgoan village of Amravati district followed by in Mozari (40.19%) Tahsil Tiwsa district Amravati. Whereas minimum per cent disease incidence (12.50%) was noticed in village Tuljapur Taluka Seloo district Wardha followed by Jambhrun Parande village in Washim district (13.93%).

In Akola district, maximum disease incidence was observed in Balapur tahsil (36.36%) during pod development stage and minimum in Sonala village (14.98%) at flowering stage. In Buldhana district, highest disease incidence (37.50%) observed in Jalgoan Jamod Tahsil whereas minimum disease incidence (18.18%) was noticed in Ambhoda village of Buldhana district during pod development stage.

In particular Yavatmal district, three tahsil surveyed namely Yavatmal, Kalamb and Babhulgoan and disease incidence noticed to those of 28.57 to 35.50%.

During the course of investigation, almost all surveyed areas concurrently noticed that JS-335 and JS-9305 cultivar were sown on farmer fields and found variation in disease incidence. These results are agreement with the findings of Kamthane and Rakh (2013) who reported

JS-335 cultivar was most susceptible to Alternaria leaf spot compared to JS-9305 as 66% disease incidence noticed on JS-335 whereas 11 % incidence recorded on JS-9305 during the survey in Marathwada region of Maharashtra state.

Table 3. Roving suvey for incidence of Alternaria leaf spot disease in Vidarbha region

S N.	District	Taluka	Village	Variety	Stage of crop	Disease Incidence
1	Amravati	Amravati	Walgaon	JS-335	Pod development	41.66
		Tiwsa	Mozari	JS-335	Pod development	40.19
		Daryapur	Yevada	JS-9305	Pod initiation	18.48
2	Washim	Washim	Jambhrun Parande	JS-9305	Pod development	13.93
		Malegaon	Brahmanwada	JS-335	Flowering	38.62
		Risod	Chinchamba	JS-335	Pod initiation	34.42
3	Akola	Akola	Sonala	JS-9305	Flowering	14.98
		Balapur	Kolsa	JS-9560	Pod development	16.79
		Balapur	Wadegaon	JS-335	Flowering	29.15
		Balapur	Balapur	JS-335	Pod development	36.36
		Barshi Takali	Sukli	JS-335	Pod development	25.33
4	Buldhana	Buldhana	Ambhoda	JS-9305	Pod development	18.18
		Jalgaon jamod	Jalgaon Jamod	JS-335	Pod development	37.50
		Nandura	Nandura	JS-335	Pod development	25.00
5	Yavatmal	Yavatmal	Yavatmal	JS-335	Pod initiation	28.57
		Kalamb	Kotha	JS-335	Pod initiation	33.33
		Babhulgaon	Veni	Local	Pod initiation	35.50
6	Nagpur	Kalmeshwar	Sawangi	JS-9305	Pod development	21.42
		Katol	Khangaon	JS-9560	Pod development	14.28
7	Wardha	Seloo	Tuljapur	JS-9305	Pod development	12.50
		Devali	Gaul	JS-335	Pod development	20.00
		Hinganghat	Kutki	JS-335	Pod development	16.66

These results are also in close agreement with Anonymous (2016) who reported maximum 36.17% incidence of Alternaria leaf spot disease in Amravati district followed by Akola (30.85%) and Buldhana



CV-JS-335 (Akola)



CV- JS-335 (Buldhana)



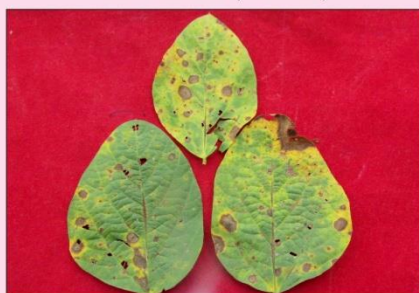
CV-JS-335 (Washim)



CV-JS-9305 (Wardha)



CV- JS-335 (Nagpur)



CV.-JS-335 (Yavatmal)



CV-JS-335 (Amravati)

Plate 1 Representative *Alternaria* Leaf spot leaf samples collected from different locations

(24.50%) district. The variation in incidence of *Alternaria* leaf spot observed during surveying places. It might be due to the prevailing conditions (like temperature, relative humidity, distribution of rainfall followed by cultural practices like sanitation and there suitable management measures) for the disease development and sufficient inoculum to cause infection in respective cultivars. Also age of crop and variety sown were more favorable for *Alternaria* leaf spot disease to become severe.

The higher incidence of *Alternaria* leaf spot was attributed to the temperature and relative humidity prevailed during the crop period which is favorable for the soybean *Alternaria* leaf spot development and spread (Gupta and Chavan, 2005).

4.2 The district wise distribution of different *Alternaria* spp. in association with *Alternaria* leaf spot of soybean

The district wise observation are recorded to know the association between *Alternaria* spp. causing *Alternaria* leaf spot of soybean in Vidarbha region and data is presented in Table 4.

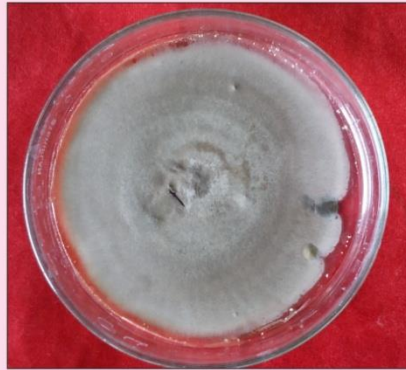
Total 440 bits of infected leaf sample of soybean *Alternaria* leaf spot subjecting for isolation, the main pathogen isolated was *Alternaria alternata* (85.92%) followed by *Alternaria tenuissima* (3.85%). This indicated that *Alternaria alternata* was the major pathogen species associated with soybean in Vidarbha region.

Among the seven districts surveyed, maximum occurrence of *Alternaria alternata* was observed in Amravati district (90.0%) followed by Akola (89.0%), Nagpur (87.50%) and Buldhana district (86.66%). Minimum occurrence of *Alternaria alternata* was observed in Wardha district (83.33%). Data in respect of *Alternaria tenuissima* occurrence, reveals that, maximum occurrence was observed in Akola (7.0%) followed by Buldhana (6.66%), Amravati (5.0%) and Nagpur (5.0%).

In Wardha and Yavatmal districts interestingly noted that there is no association was observed of *Alternaria tenuissima* in soybean *Alternaria* leaf spot as only *Alternaria alternata* association was observed in infected bits in respective districts.

Table 4. Pathogen association with soybean *Alternaria* leaf spot

SN.	District	Village	No. of bits used for isolation	Fungi obtained				
				<i>Alternaria alternata</i>		<i>Alternaria tenuissima</i>		Other (%)
				No. of bits yielded fungi	Occurrence (%)	No. of bits yielded fungi	Occurrence (%)	
1	Amravati	Walgaon	20	18	90	1	5	1(5)
		Mozari	20	17	85	2	10	1(5)
		Yevada	20	19	95	00	00	1(5)
Average mean (%)					90.0		5	5
2	Washim	Jambhrun Parande	20	16	80	1	5	3(15)
		Brahmanwada	20	17	85	00	00	3(15)
		Chinchamba	20	17	75	1	5	4(20)
Average mean (%)					80.0		3.33	16.33
3	Akola	Sonala	20	18	90	2	10	0(00)
		Kolsa	20	17	85	2	10	1(5)
		Wadegaon	20	19	95	0	00	1(5)
		Balapur	20	18	90	1	5	1(5)
		Sukli	20	17	85	2	10	1(5)
Average mean (%)					89.0		7.00	4.00
4	Buldhana	Ambhoda	20	19	95	00	00	1(5)
		Jalgoan Jamod	20	17	85	2	10	1(5)
		Nandura	20	16	80	2	10	2(10)
Average mean (%)					86.66		6.66	6.66
5	Yavatmal	Yavatmal	20	18	90	00	00	2(10)
		Kotha	20	16	80	00	00	4(20)
		Veni	20	17	85	00	00	3(15)
Average mean (%)					85.00		00	15
6	Nagpur	Sawangi	20	17	85	1	5	2(10)
		Khangaon	20	18	90	1	5	1(5)
Average mean (%)					87.50		5	7.5
7	Wardha	Tuljapur	20	16	80	00	00	4(20)
		Gaul	20	17	85	00	00	3(15)
		Kutki	20	17	85	00	00	3(15)
Average mean (%)					83.33		00	16.66
Vidarbha (%)			440	379	85.92	18	3.85	10.16



Pure culture



Infected leaf sample



Growth on slants



Conidia of *A. alternata*



Growth on broth



Conidia of *A. alternata*

Plate 2. Pure culture and microscopic view of *Alternaria alternata*



Pure culture



Infected leaf sample



Growth on slants

Plate 3 Pure culture of *A. tenuissima*

The per cent association of *Alternaria spp.* varied from locality to locality might be due to cropping pattern, environmental conditions and precise specific species inoculums buildup. Moreover, intensive occurrence of this fungus over the last couple of years could be due to monocropping, same variety use on same piece of land, low rate of seed exchange, poor sanitation measures and climatic conditions prevailed in Vidarbha region.

These results were in conformity with Kale and Ukesh (2015) who reported that genera *Alternaria alternata* predominant in Amravati, Washim and Akola districts. Gupta and Chavan (2005) reported that *Alternaria alternata* and *Alternaria tenuissima* is most associated pathogenic fungi in Alternaria blight of soybean in India.

Earlier workers those of Sinclair and Backman, (1989); Baird *et al.* (2001) and Jasnic *et al.* (2011) confirms the *Alternaria alternata* and *Alternaria tenuissima* are most frequent species parasites of leaf, pods and soybean seeds. From present investigation, valid results emerged *i.e.* the higher association of *Alternaria alternata* in soybean leaf spot in particular in Vidarbha region. This indicated that *Alternaria alternata* was the major pathogen associated with soybean Alternaria leaf spot in Vidarbha region. This could be useful for academic studies and management of this disease.

4.3 Pathogenicity Test and Symptoms

Pathogenicity

The virulence of a field isolate of *Alternaria alternata* and *A. tenuissima* was tested by inoculating 3 weeks old plants (cv. JS-335) by spraying them with conidial suspensions (1×10^6 spores/ml) by hand sprayer, inoculated plants were immediately placed in a shade net green house for disease development where > 80 % relative humidity and $27 \pm 1^\circ\text{C}$ temperature maintained. The old leaf showed typical leaf spot symptoms after one week of inoculation.

Table 5: Pathogenicity test of *Alternaria alternata*

SN.	District	Villages	Isolate designation	PDI
1	Amravati	Walgaon	WAL ALTA	32.18
		Mozari	MOZ ALTA	16.15
2	Washim	Jambhrun Parande	JAM ALTA	14.16
		Chinchamba	CHIN ALTA	19.12
3	Akola	Sonala	SON ALTA	14.19
		Kolsa	KOL ALTA	12.15
		Balapur	BAL ALTA	18.32
		Sukli	SUK ALTA	20.22
4	Buldhana	Ambhoda	AMB ALTA	11.10
		Jalgoan Jamod	JAL ALTA	13.56
		Nandura	NAN ALTA	16.19
5	Yavatmal	Yavatmal	YAV ALTA	20.28
		Kotha	KOT ALTA	15.10
		Veni	VEN ALTA	13.04
6	Nagpur	Sawangi	SAW ALTA	21.24
		Khangaon	KHAN ALTA	9.27
7	Wardha	Tuljapur	TUL ALTA	16.23
		Gaul	GAU ALTA	10.18
		Kutki	KUT ALTA	16.15
8	Control			0.0

The pathogenicity studies showed varied degree of per cent infection with respect to *A. alternata* and *A. tenuissima* isolates (Table 5 and 6). Infection index ranged between 9.27 to 32.18 % for different isolates of *A. alternata* and the maximum being with isolate WAL ALTA (32.18%) followed by SAW ALTA (21.24%). Relatively lower per cent infection index (9.27%) was observed with KHAN ALTA (Table 5, Plate 4).



Control



Inoculated

Plate 4. Pathogenicity test of *Alternaria alternata*

Table 6: Pathogenicity test of *Alternaria tenuissima*

SN.	District	Villages	Isolate designation	PDI
1	Amravati	Walgaon	WAL ALTENU	11.13
		Mozari	MOZ ALTENU	10.10
		Yevada	YEV ALTENU	12.16
2	Washim	Jambhrun Parande	JAM ALTENU	15.12
		Brahmanwada	BRAH ALTENU	9.36
		Chinchamba	CHIN ALTENU	8.42
3	Akola	Sonala	SON ALTENU	10.11
		Kolsa	KOL ALTENU	7.68
		Wadegaon	WAD ALTENU	14.36
		Balapur	BAL ALTENU	12.22
		Sukli	SUK ALTENU	11.10
4	Buldhana	Jalgoan Jamod	JAL ALTENU	13.37
		Nandura	NAN ALTENU	14.52
5	Nagpur	Sawangi	SAW ALTENU	12.32
		Khangaon	KHAN ALTENU	10.34
6	Control			0.0

In comparison to *A. alternata*, *A. tenuissima* isolates shown minimum PDI (Table 6) on cv. JS-335. Maximum infection index recorded by isolate JAM ALTENU (15.12%) while lowest (7.68%) registered in KOL ALTENU isolate.

All the isolates of *A. alternata* and *A. tenuissima* were found pathogenic to the cv. JS-335. From these findings, it can be concluded that *A. alternata* and *A. tenuissima* pathogenic to tested cultivar.

The above findings are on the similar lines observed by earlier workers those of Sinclair and Backman, (1989) and Kale and Ukesh (2015) who reported that soybean plants are susceptible to *Alternaria*

alternata and *Alternaria tenuissima* infection. The pathogenicity of the fungus earlier verified and reported by Khan and Sinclair (1992)

The above results were also in agreement with Kamthane and Rakh (2013) proved the pathogenicity on cultivar JS-335 on soybean by inoculating spore suspension (10^6 spores/ml) grown on PDA. The symptoms appeared 8-9 days after inoculation. Jasnic *et al.* (2011) continue also confirms the present investigation as they have reported variation among different isolates of *A. tenuissima* on the basis of virulence collected from different locations. Difference in the level of virulence of isolates may be due to pathotype, genetic recombination and mutation as isolates were collected from various locations might have influenced their genetic makeup.

The organism was re-isolated on the PDA and morphological, cultural and microscopic observations made were found similar to that of the organism isolated from naturally infected soybean plants. Thus the test pathogen was confirmed as *Alternaria alternata* and *Alternaria tenuissima* and pathogenicity of the test pathogen was also proved.

Taking into consideration of higher association of *A. alternata* species in Vidarbha region and elevated PDI in isolate (WAL ALTA) during the pathogenicity test, this isolate used for further studies.

Symptoms

Symptoms appeared on the leaves after 8 days of inoculation. Initial symptoms were production of small water soaked spots brown in colour on the leaves. Later the spots develop into dark brown shaped with characteristic concentric rings in the center to produce a target board effect and the colour of the spots changed from brown to dark brown. The lesions expand and may combine to yield larger dead areas on the leaves. Infected leaves eventually dry out and fall.

4.4 Efficacy of fungicides against *Alternaria alternata* by poisoned food technique

Efficacy of 11 fungicides at respective concentration was tested *in-vitro* by following poison food technique for mycelial growth of *Alternaria alternata*.

Table 7. Effect of fungicides on radial mycelial growth of *Alternaria alternata*

SN	Fungicides	Concentrations	Mean radial mycelial growth (mm) * 7 DAI	Mycelial inhibition (%)
1	Mancozeb	0.2	27.67	62.97
2	Carbendazim	0.1	62.50	16.36
3	Mancozeb + Carbendazim	0.2	27.00	63.86
4	Hexaconazole	0.1	0.00	100.0
5	Propiconazole	0.1	0.00	100.0
6	Coper Oxchloride	0.3	55.50	25.73
7	Propineb	0.2	0.00	100.0
8	Thiophanate Methyl	0.2	55.67	25.50
9	Tebuconazole+Trifloxystrobin	0.1	15.67	79.03
10	Chlorothalonil	0.2	20.00	73.23
11	Cymoxanil+Mancozeb	0.1	0.00	100.0
12	Control	-	74.73	
	F ' test	-	Sig.	-
	SE(m)±	-	0.89	-
	CD(P=0.01)	-	2.59	

*Average of 3 replication.

* DAI- Day after inoculation

Results presented in table 7, plate 5 showed that the effect of fungicides on radial mycelial growth of *Alternaria alternata*. After 7 days of inoculation hexaconazole, propiconazole, propineb and cymoxonil + mancozeb was found most effective in arresting growth of *Alternaria alternata* as complete (100%) inhibition observed in respective tested

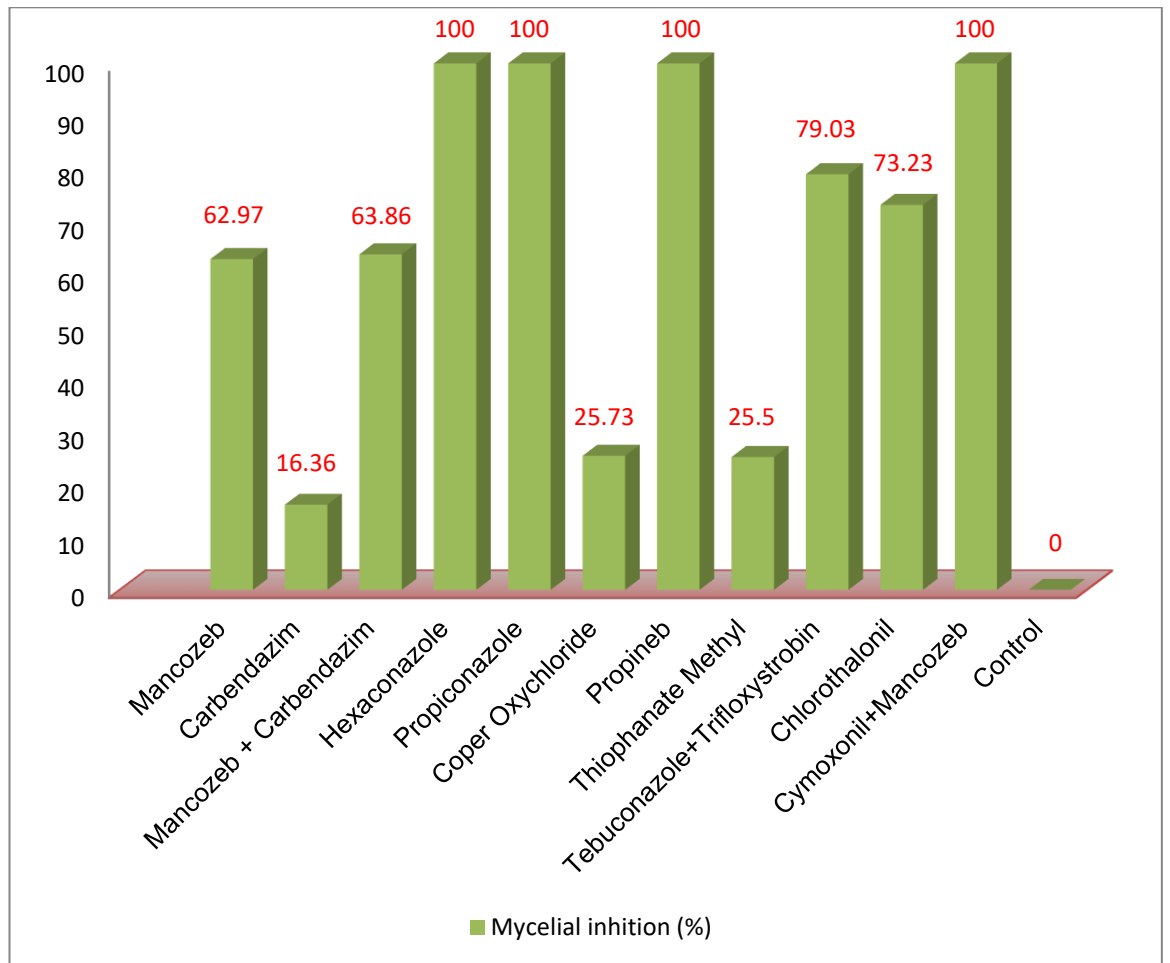


Fig. 2 Effect of fungicides on radial mycelial growth of *Alternaria alternata*

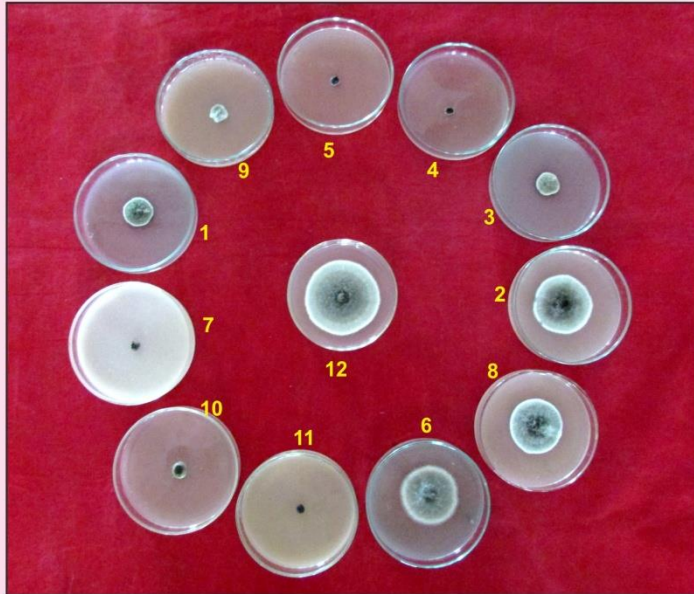


Plate 5a. Efficacy of fungicides against *Alternaria alternata*



Plate 5b. Efficacy of botanicals against *Alternaria alternata*

fungicides. Tebuconazole + Trifloxystrobin, Chlorothalonil and mancozeb next in order to restrict the growth by 79.03, 73.23 and 62.97% respectively. Carbendazim, Thiophanate Methyl and Copper-oxychloride were found least effective as recorded the 16.36, 25.50, and 25.73 % inhibition. Maximum radial growth was recorded in control plate (74.73 mm).

The present findings are in agreement with Thaware *et al.* (2010), Maheswari and Krishna (2013) and Nidhika Rani (2018) who reported that propiconazole and hexaconazole was most effective fungicides against *Alternaria alternata*. Singh and Majumdar (2002) also reported that propiconazole was the most effective fungicide in controlling *A. alternata* by 100% in 8 day after inoculation.

No related information could be traced during literature hunt in respect to propineb and cymoxonil + mancozeb efficacy against *A. alternata*. Thus, it provides additional information as effective fungicides for management of *A. alternata*.

Under laboratory studies, relatively higher 79.03, 73.23 and 62.97% per cent inhibition recorded in Tebuconazole + Trifloxystrobin, Chlorothalonil and mancozeb respectively. This finding are almost similar to those of Hiremath and Sundaresh (1985); Amaresh and Nargumd (2002) and Jakatimath *et al.* (2017) who reported the judicious inhibition of *Alternaria alternata* by tebuconazole, chlorothalonil and mancozeb. In present investigation carbendazim, and copper-oxychloride were found least effective as recorded the minimum per cent inhibition against *A. alternata*. These findings seem to be in contrast with those of Kantwa *et al.* (2014) who recorded copper oxychloride and carbendazim exhibit maximum mycelial inhibition. Thiophanate Methyl also found less effective in *in vitro* studies in present investigation with 16.36% inhibition of *A. alternata*. Pareek *et al.* (2012) also observed that thiophanate methyl was less effective against *A. alternata*.

4.5 Efficacy of botanicals against *Alternaria alternata* by poisoned food technique

Eleven plant extract were evaluated against *Alternaria alternata* isolated from soybean leaves by poisoned food technique and data presented in table 8.

Table 8. Efficacy of botanicals against *Alternaria alternata* by poisoned food technique

SN	Botanicals	Conc. (%)	Mean radial mycelial growth (mm)*	Per cent mycelial inhibition (%)
1	Neem leaf extract	10	44.53	47.34
2	Parthenium Leaves extract	10	75.07	11.23
3	Ghaneri leaves extract	10	54.43	35.63
4	Mehandi leaves extract	10	50.83	39.89
5	Datura leaves extract	10	63.00	25.50
6	Garlic clove extract	10	10.57	87.50
7	Nilgiri leaves extract	10	64.67	23.53
8	Beshram leaves extract	10	68.20	19.35
9	Ginger rhizome extract	10	56.67	32.99
10	Turmeric rhizome extract	10	64.67	23.53
11	Onion bulb extract	10	47.80	43.47
12	Control	-	84.57	
	F' test	-	Sig.	-
	SE(m)±	-	0.83	-
	CD(P=0.01)		2.41	

*Average of four replication

The results presented in the table 8, plate 5 indicated that all the tested botanicals showed significantly differences compared with

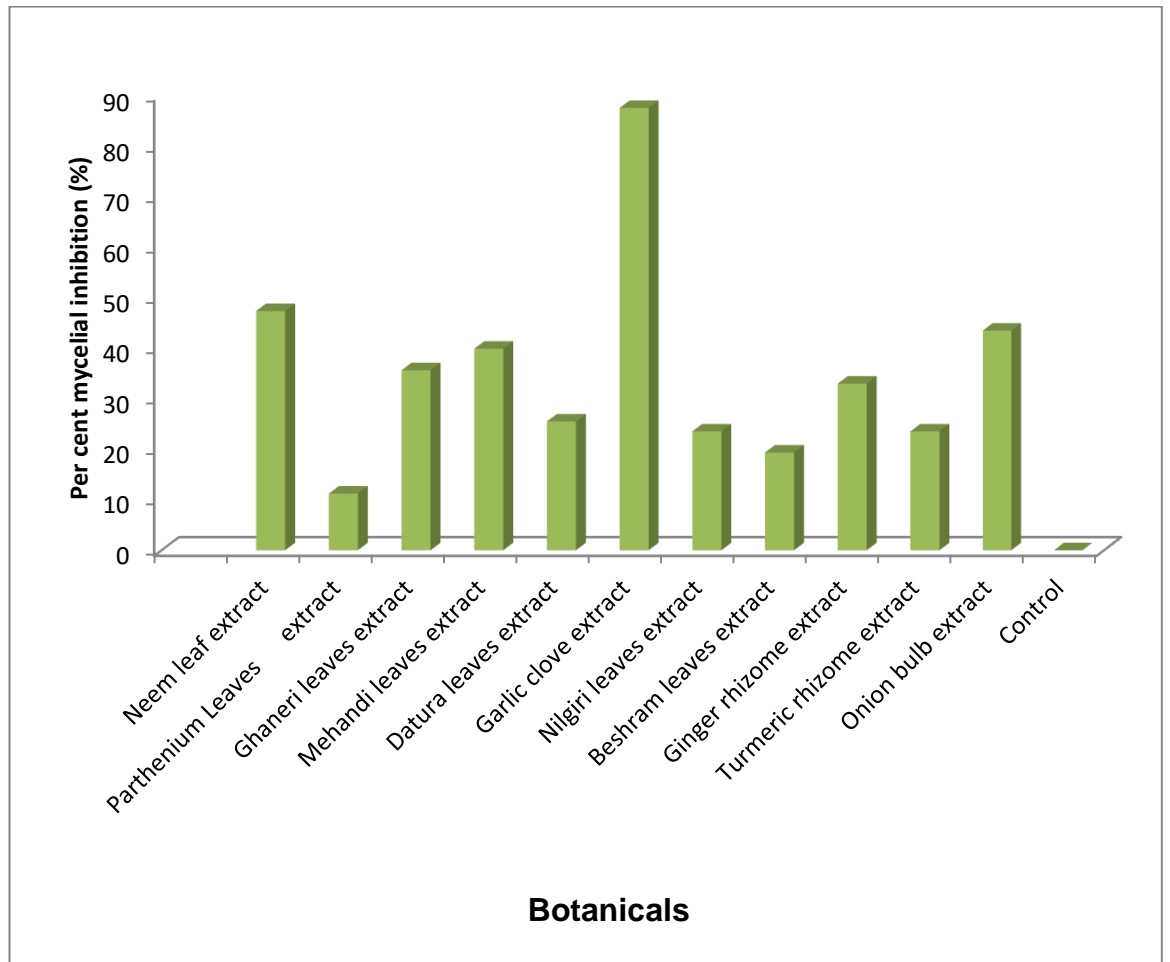


Fig. 3 Efficacy of botanicals against *Alternaria alternata* by poisoned food technique

control. Among the plant extracts garlic clove extracts @ 10% recorded maximum inhibition (87.50%) of mycelial growth of test fungus and was significantly superior to rest of the treatments. This was followed by Neem leaf extract and Onion bulb extract recorded 47.34 and 43.47 per cent mycelial inhibition of *A. alternata*. Rest of the plant extract viz. Parthenium Leaves extract, Ghaneri leaves extract, Mehandi leaves extract, Datura leaves extract, Nilgiri leaves extract, Beshram leaves extract, Ginger rhizome extract and Turmeric rhizome extract recorded per cent inhibition in the range of 11.23 to 39.89% which least effective against test pathogen. These results are almost similar to those of Bhosale *et al.* (2014) who observed the mycelial inhibition of *Alternaria alternata* due to Garlic clove extract by 80.30% in soybean. Singh *et al.* (2014) also observed the effectiveness of garlic and neem extract against *Alternaria alternata*. Likewise Thaware (2010) moreover reported that the garlic clove extract showed maximum mycelial inhibition (63%) followed by neem (33%), karanj (26.66%) and tulsi (27.77%) against *A. alternata*.

The findings of Nidhika Rani *et al.* (2018) are on the similar line of the present results; while testing the different efficacy of botanicals they reported that garlic clove extract @ 10% concentrations exhibited the maximum (84.31%) inhibition against *Alternaria alternata* whereas 82.18% inhibition of mycelial growth was observed in case of *Alternaria tenuissima* followed by onion and neem. The fungi toxicity of plant extracts in the present study might be due to antifungal metabolites present in different plant species.

4.6 Efficacy of bio-agents against *Alternaria alternata* by dual culture technique

Antagonistic activity of bio-control agents namely *Trichoderma asperellum*, *Trichoderma atroviride*, *Trichoderma harzianum*, *Pseudomonas fluorescence* and *Bacillus subtilis* were investigated by using dual culture technique.

Table 9. Efficacy of bio-agents against *Alternaria alternata* by dual culture technique

SN	Bioagents	Mean radial mycelial growth (mm)*	Per cent mycelial inhibition (%)
1	<i>Trichoderma asperellum</i>	19.97	76.55
2	<i>Trichoderma atroviride</i>	30.10	64.65
3	<i>Trichoderma harzianum</i>	17.33	79.65
4	<i>Pseudomonas fluorescence</i>	26.67	68.68
5	<i>Bacillus subtilis</i>	44.03	48.30
6	Control	85.17	-
	F' test	Sig.	-
	SE(m)±	0.48	-
	CD (P=0.01)	2.06	-

*Average of four replication

All the bio-agents tested showed clear significant effect compared with were control (Table 9, Plate 6). Antagonist *Trichoderma harzianum* and *Trichoderma asperellum* gave the best effect against *Alternaria alternata* forming maximum percent mycelial inhibition i.e. 79.65% and 76.55 % and decreased the mycelial growth from 85.17 to 17.33 and 19.97 mm respectively. *Pseudomonas fluorescence* was next best recorded (68.68%) inhibition while the least mycelial inhibition was observed in case of *Bacillus subtilis* (48.30%).

In current experiment fungal antagonist found effective than bacterial known antagonist. The present findings are conformity with the results of Jakatimath *et al.* (2017) who reported that *Trichoderma spp.* was found most effective against *Alternaria alternata* as compared *Pseudomonas fluoroscens* and *Bacillus subtilis*. These results are also in accords with Thaware *et al.* (2010) who reported effectiveness of *Trichoderma harzianum* and *Trichoderma viride* against mycelial growth of *Alternaria alternata* by 85.88% and 81.88% respectively. The similar

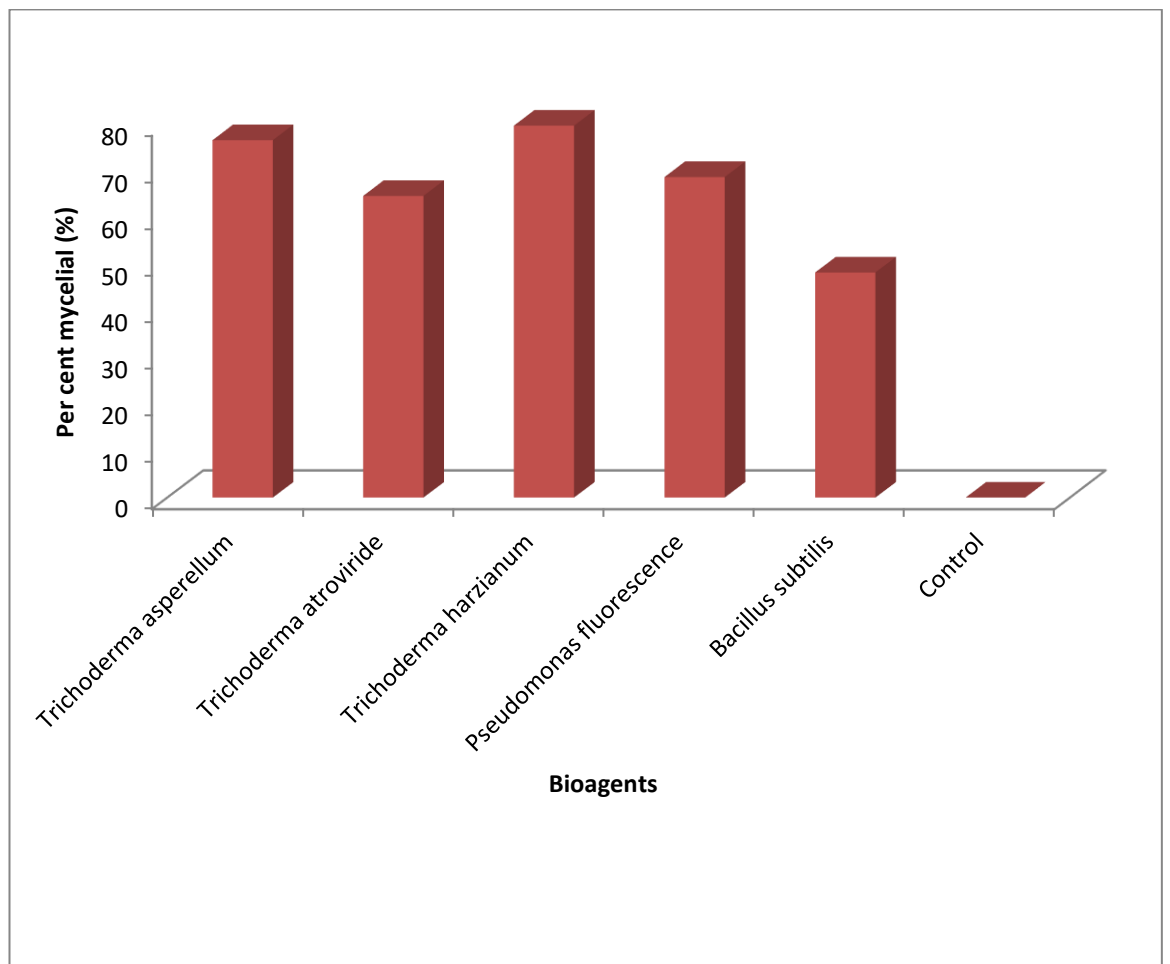
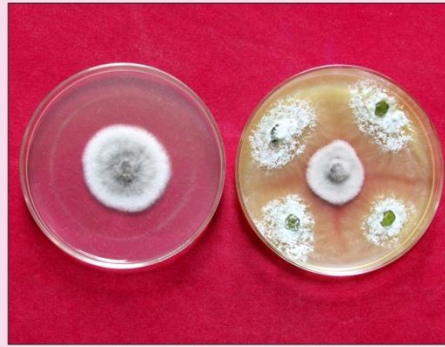


Fig. 4 Efficacy of bio-agents against *Alternaria alternata* by dual culture technique



Trichoderma asperellum



Trichoderma atroviride



Trichoderma harzianum



Pseudomonas fluorescens



Bacillus subtilis



Control

Plate 6 Bioefficacy of bio-agents against *Alternaria alternata*

observation was also reported by Chaitali (2014) who recorded that *Trichoderma harzianum* was most effective against *Alternaria* fungus with significant mycelial inhibition (83.3%) followed by *Trichoderma viride* (81.4%), *Pseudomonas fluorescense* (66%) and *Bacillus subtilis* (67.8%).

The inhibition of fungal growth due to *Trichoderma spp.* may have been due to secretion of extracellular cell degrading enzymes such as chitinase α -1, 3-glucanase, cellulose and lectin, which may have helped myco-parasites in the colonization of their host. The inhibition of pathogen may also be attributed to the production of secondary metabolites by antagonists such as glioviridin, viridian and gliotoxin (Behairy *et al.*, 2014).

4.7 Screening of varieties against *Alternaria* leaf spot of soybean

Thirty three genotype along with checks were screened against *Alternaria* leaf spot of soybean under natural field condition during kharif season and data presented in Table 10, Plate 7.

Table 10. Screening of soybean genotypes against *Alternaria* leaf spot

Sr. No	Varieties	Percent disease index		Reaction	Yield /plot (Kg/ha)
		30 DAS	65DAS		
1	AMS 2014-C	00	9.16	HR	975.31
2	TAMS-38 ©	00	20.40	MR	1051.85
3	AMS-105	00	14.20	MR	1008.64
4	AMS-MB-5-18	00	16.20	MR	1358.02
5	JS-20-87	00	10.79	HR	1150.61
6	JS-20-69 ©	00	12.39	MR	1177.77
7	AMS-475	00	9.10	HR	1153.08
8	JS-20-29 ©	00	12.72	MR	991.35
9	PS-1569	00	17.85	MR	1017.28
10	AMS-2014-B	00	7.44	HR	1123.45
11	JS-72-44 ©	00	60.10	S	855.55
12	NRC-117	00	8.33	HR	1125.92
13	SL-20-74	00	10.31	HR	1301.23
14	AMS-56	00	18.30	MR	1258.02

15	JS-9305 ©	00	10.93	HR	1049.38
16	AMS-1001	00	15.10	MR	1333.33
17	MACS-1460	00	38.42	MS	969.13
18	AMS-1004	00	12.50	MR	1301.60
19	DSB-28-3	00	10.51	HR	1260.00
20	KDS-869	00	13.56	MR	1382.71
21	AMS-102	00	14.98	MR	866.66
22	VLS-58	00	35.16	MS	818.51
23	RVS-2010-1	00	10.17	HR	1298.76
24	JS-335 ©	00	58.50	S	853.08
25	AMS-38-24	00	14.40	MR	875.30
26	AMS-MB-5-19	00	10.84	HR	1308.63
27	JS-75-46 ©	00	59.40	S	762.96
28	AMS-77	00	16.50	MR	816.04
29	JS-20-94	00	35.10	MS	980.71
30	AMS-243	00	38.62	MS	975.31
31	AMS-32	00	4.76	HR	1051.85
32	AMS-353	00	23.33	MR	808.64
33	AMS-358	00	11.10	HR	1158.02

Data shows that, among 33 genotype including checks of soybean, JS-72-44, JS-75-46, and JS-335 shows maximum per cent disease incidence *i.e.* 60.10, 59.40 and 58.50% respectively. Lowest per cent disease incidence was recorded in AMS-32 (4.76%).

None of the tested genotype showed immune reaction; however 12 genotype namely (AMS-2014-C, JS-20-87, AMS-475, AMS-2014-B, NRC-117, SL-20-74, JS-9305, DSB-28-3, RVS-2010-1, AMS-MB-5-19, AMS-32 and AMS-358) exhibited Highly Resistant (HR) reaction while 14 genotype shown Moderately Resistant (MR) reaction and 4 genotype *viz.* MACS-1460, VLS-58, JS-20-94 and AMS-243 exhibited Moderately Susceptible (MS) reaction. Rest of genotype including checks *viz.* JS-72-44, JS-335 and JS-75-46 shown Susceptible (S) reaction.



Plate 7. General view of experimental field

Data in respect of yield, highest yield was recorded in genotype KDS-58-69 (1382 kg/ha) followed by AMS-MB-5-18 (1358.02 kg/ha), AMS-1001 (1333.33 kg/ha), AMS-MB-5-18 (1308.63 kg/ha) and SL 20-74 (1301.23 kg/ha).

The work on identification of multiple disease resistance sources in cultivable and wild soybean has been undertaken many workers like Anonymous (2015), Ingle. *et al.* (2016) and Anonymous (2016).

In current investigation, entries showed varied reaction ranging from HR to S. This might be due to the availability of inoculum or differential interactions of host genotype with different varieties.

Mahesha *et al.* (2009) evaluated 204 soybean genotypes under natural and laboratory conditions against major disease and reported several genotypes having multiple resistance source.

The present finding are also in accordance with the Anonymous (2016) who reported that maximum per cent disease incidence on JS-72-44 (44.15%) and JS-75-46 (39.89%) while mild incidence recorded on JS-9305.

In present investigation genotype viz. KDS-58-69, AMS-MB-5-18, AMS-1001, AMS-MB-5-18 and SL 20-74 exhibited Highly Resistant and Modertaley Resistant reaction with good yield potential. Thus these resistant sources of soybean genotype may have scope in future for breeding in development of variety or in integrated disease management programme.

CHAPTER V

SUMMARY AND CONCLUSIONS

Soybean crop suffers from a number of foliar pathogens among these leaf spot incited by *Alternaria spp.* reported to be the most prevalent and widespread disease. *Alternaria alternata* and *Alternaria tenuissima* causes Alternaria leaf spot disease in soybean and causes yield losses.

Survey for Alternaria leaf spot disease incidence was carried out in Vidarbha region and per cent disease incidence was recorded in the range of 12.50- 41.66%. In surveyed areas maximum disease incidence was recorded in Walgoan (41.66%) in Amravati district and minimum disease incidence was recorded in Tuljapur (12.60%) in Wardha district field.

Infected leaf samples were brought in laboratory and pathogen isolated confirmed as *Alternaria alternata* and *Alternaria tenuissima*.

Efficacy of 11 fungicides was tested *in-vitro* against *Alternaria alternata* employing poison food technique. The treatment hexaconazole, propiconazole, propineb and cymoxanil+Mancozeb found effective and recorded significantly highest inhibition of mycelial growth (100%) at 0.1, 0.1, 0.2 and 0.1% concentration respectively. The least inhibition was recorded in Carbendazim (16.36%) at 0.1% concentration.

Efficacy of 11 botanicals was studied *in-vitro* against *alternaria alternata* by using poison food technique. The treatment of garlic clove extract found effective and recorded significantly maximum inhibition of mycelial growth (87.50%) at 10% concentration. The least inhibition was recorded in Parthenium leaves (11.23%) at 10% concentration.

In-vitro evaluation of per cent inhibition of mycelial growth of *Alternaria alternata* against bioagent *Trichoderma harzianum* by dual culture resulted in 79.65% inhibition.

During the study, screening of the advance soybean genotypes tested under natural field condition. Under natural field

conditions, out of 33 entries tested 12 genotype namely (AMS-2014-C, JS-20-87, AMS-475, AMS-2014-B, NRC-117, SL-20-74, JS-9305, DSB-28-3, RVS-2010-1, AMS-MB-5-19, AMS-32 and AMS-358) exhibited Highly Resistant (HR) reaction. While 14 genotype shown Moderately Resistant (MR) reaction and five genotype viz. MACS-1460, VLS-58, JS-20-94 and AMS-243 exhibited Moderately Susceptible (MS) reaction. Rest of genotype including checks viz. JS-72-44, JS-335 and JS-75-46 shown Susceptible (S) reaction.

Conclusions

Per cent disease incidence was recorded in the range of 12.50- 41.66% in Vidarbha region. In survey area maximum disease incidence was recorded in Walgoan (41.66%) in Amravati district and minimum disease incidence was recorded in Tuljapur (12.60%) in Wardha district.

Among the seven districts surveyed, maximum occurrence of *Alternaria alternata* species was observed in Amravati district (90%) followed by Akola (89%), Nagpur (87.5%) and Buldhana district (86.66%). Least per cent association of *Alternaria tenuissima* observed in surveyed area.

Hexaconazole(0.1%) Propiconazole (0.1%), Propineb (0.2%) and Cymoxanil+ Mancozeb (0.%) were found to be most effective fungicides over all other treatments for arresting the mycelial growth (100%) of *Alternaria alternata*.

Among botanicals, maximum mycelial inhibition was recorded in Garlic clove extracts.

Biocontrol agents, *Trichoderma hurzianum*, *Trichoderma asperellum* was found most effective for arresting mycelial growth of *Alternaria alternata*.

Among the 33 genotype 12 genotype namely (AMS-2014-C, JS-20-87, AMS-475, AMS-2014-B, NRC-117, SL-20-74, JS-9305, DSB-28-3, RVS-2010-1, AMS-MB-5-19, AMS-32 and AMS-358) exhibited Highly Resistant (HR) reaction, While 14 genotype shown Moderately Resistant

(MR) reaction and 4 genotype viz. MACS-1460, VLS-58, JS-20-94 and AMS-243 exhibited Moderately Susceptible (MS) reaction. Rest of genotype including checks viz. JS-72-44, JS-335 and JS-75-46 shown Susceptible (S) reaction.

KDS-58-69, AMS-MB-5-18, AMS-1001, AMS-MB-5-18 and SL 20-74 exhibited Highly Resistant and Modertaley Resistant reaction with good yield potential.

CHAPTER VI

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(ZADE SAMIR BABARAO)

APPENDIX- I

Composition of potato dextrose agar (PDA)

Peeled potato	- 200 g
Agar-agar	- 20 g
Dextrose	- 20 g
Distilled water	- 1000 ml

Composition of potato dextrose broth (PDB)

Peeled potato	- 200 g
Dextrose	- 20 g
Distilled water	- 1000 ml