ETIOLOGY AND MANAGEMENT OF BLIGHT OF MARIGOLD (*Tagetes erecta* L.) CAUSED BY *Alternaria alternata* (Fr.) Keissler

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ETIOLOGY AND MANAGEMENT OF BLIGHT OF MARIGOLD (*Tagetes erecta* L.) CAUSED BY *Alternaria alternata* (Fr.) Keissler

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CERTIFICATE

This is to certify that the thesis entitled **"ETIOLOGY AND MANAGEMENT OF BLIGHT OF MARIGOLD (*Tagetes* spp.) caused by *Alternaria alternata* (Fr.) Keissler"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **PLANT PATHOLOGY** to the University of Horticultural Sciences, Bagalkot, is a record of bonafide research work carried by **MISS. ARCHANA, A. M., ID No. UHS17PGM905** under my guidance and supervision and that no part of the thesis has been submitted for the award of any degree, diploma, associateship, fellowship or other similar titles.

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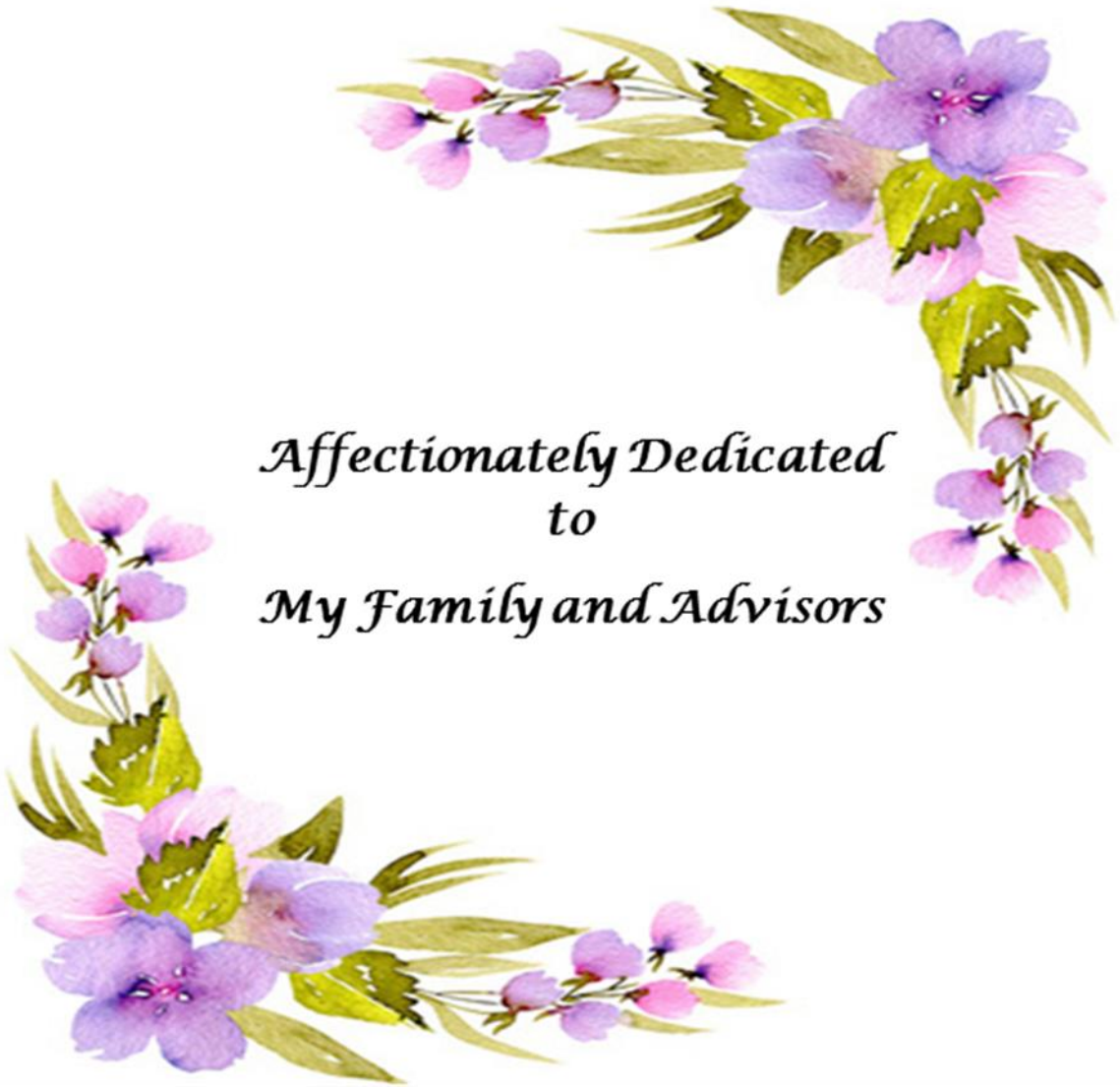
Affectionately Dedicated
to
My Family and Advisors

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1. INTRODUCTION

Marigold (*Tagetes* spp.) is one of the important flower crops grown commercially in different parts of India. The flower was introduced in India from Portugal during 16th century, since then it has been naturalized in different agro climatic zones. Although there are about 33 species of the genus *Tagetes*, which belongs to Asteraceae (Compositae) family, five species have been introduced into the Indian gardens *viz. Tagetes erecta* L. (African Marigold), *Tagetes minuta* L. (*Tagetes glandulifera* Schrank), *Tagetes patula* L. (French Marigold), *Tagetes lucida* Cav. (Sweet-Scented Marigold) and *Tagetes tenuifolia* Cav. (Striped Marigold) (Rydberg, 1915). Out of these, *T. erecta* and *T. patula* are the dominant species native to North and South America.

Marigold is cultivated in a wide range of soils except water logged conditions. However, soil with good characters *viz.*, deep fertile, friable with good water holding capacity, well drained and nearer to neutral in reaction (pH 7.0 – 7.5) is most desirable. It requires a mild climate for luxuriant growth and flowering. Moderate climate with optimum temperature during the growing period (14.5-28.6 °C) greatly improves flowering while higher temperature (26.2-36.4 °C) adversely affects flower production.

In India, marigold ranks first among the loose flowers. The total area under marigold cultivation in India is 66.13 thousand ha with an annual production of 603.18 thousand metric tonnes. It is mainly cultivated in the states of Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh and Haryana. In Karnataka, Chamrajanagar, Haveri, Mysore, Bellary and Belgaum are the major growing districts of marigold (Anon., 2017). The area of marigold in Karnataka is 9830 ha and production is 87.34 thousand metric tonnes (Anon., 2016).

Marigold gained popularity because of its adaptability to various soil and climatic conditions and longer blooming period and more economic returns to farmers. Flowers of marigold are used on many festive occasions like marriages, religious ceremonies, and social functions. This flower has a wide spectrum of attractive colors, shapes, and sizes and has good keeping quality. The leaves and flowers possess medicinal value and it shows pharmacological properties *viz.*, anti-microbial,

insecticidal, anti-bacterial, nematicidal, wound healing, anti- oxidant and larvicidal activity (Tripathy and Gupta, 1991). It has also been found beneficial to control nematodes population when planted as intercrop and also effective as organic manure (Polthance and Yamazaki, 1996). Mainly they are known to control root knot nematodes, lesion nematodes, *Pratylenchus* spp. and *Meloidogyne* spp. infesting crop plants. It can be used as a cover crop or in a crop rotation as they produce a substance called alpha-terthienyl. The nematicidal compound (alpha-tertheinyl) is released by active, living marigold roots, because exposure to near UV light inactivates the alpha tertheinyl when it is taken out of the soil. Thus there is no benefit in amending a planting site with marigold extracts of homogenized plant parts.

The *Tagetes* oil has been mainly exploited for the intensifying of high quality perfumes. *Tagetes minuta* is considered as the finest source of antique essential oil compared to other species of the genus and its oil possesses a very strong and penetrating odor that makes a good market in the perfumery world (Chopra *et al*., 1963). Yellow and orange colors in marigold have lutein pigment which is often added in poultry diet to intensify the yellow color of egg yolks and broiler skin (Gupta, 1997; Sreekala and Raghava, 2003). The potential source of active products are recognized by the *Tagetes i.e.* carotenoids as food colorants, feed additives and possess anticancer and anti-ageing effects. The essential oil is known for antimicrobial and insecticidal properties, thiophenes have considerable biocidal activity and flavonoids having pharmacological properties (Gupta and Vasudeva, 2012).

With an increase in area, the crop is gradually becoming susceptible to unlimited soil, seed and air borne pathogens. Marigold is infected by many fungal and bacterial diseases namely leaf spot and flower blight (*Alternaria* sp.), collar rot (*Phytophthora* sp.*; Pythium* sp), wilt (*Fusarium oxysporum*), Cercospora leaf spot (*Cercospora melalopotamica*), damping off (*Pythium* sp), powdery mildew (*Oidium spp.*), bacterial wilt (*Ralstonia solanacearum*), Botrytis flower blight (*Botrytis sp.*) (Sohi, 1983; Pawar, 1971). Among these Alternaria leaf spot and flower blight caused by *Alternaria alternata* (Fr.) Keissler is one of the most destructive and economically important disease and causes up to 50-60 % economic loss in flower yield (Cotty *et al.*, 1983) and severity of this disease in cv. African marigold was to the tune of 60 % (Sen, 1996).

Alternaria sp causes early blight of *T. erecta* and produces phytotoxic metabolites (alternaric acid) which have an adverse effect on cell viability, fresh mass and number of cells, induced reactive oxygen species accumulation, lipid peroxidation and DNA damage on *T. erecta* cell suspension culture; this is related to the pathogenic mechanism and to phytotoxins (Gamboa-Angulo *et al*. 2001). The conidia of the pathogen are produced on lesions of matured leaves. Spore production starts in ten days after the first symptoms appear and it continue up to fifty days. *Alternaria alternata* conidia disperse *via* air currents and their release from the lesions is triggered by rainfall or even just a sudden drop of humidity. The landed conidia germinate within over night with dew and enter through the stomata or penetrate directly through the leaf surface by forming appressorium, infecting the leaf within 12 hours and then infection spreads to all parts of the plant (Wiest *et al.,* 1987).

The disease results in the formation of small, brown, necrotic spots on most of the foliage. The spots gradually enlarge, become irregular in shape, or remain circular, and with concentric rings or zones. In the later stages of infection, the spots coalesce, and the leaves withere, dry, and fall off from the plants. On blooming, the flower axis and head are attacked severely turning the original yellow or orange color of the flowers to deep brown or blackish on formation of spores (Hotchkiss and Baxter, 1983; Karlatti *et al*., 1989).

A major research work has been done on this disease, yet there are many obscured aspects of the disease, which need immediate attention. Most of the work done so far on *A. alternata* causing leaf spot in marigold pertains to symptamatology, environmental factors favoring for infection and development of disease and use of fungicides in management of the disease. But very little work has been done on systematic survey in Northern Karnataka, influence of various culture media on growth of the pathogen as well as sporulation and colony characteristics and integrated management of the disease.

Hence, keeping in view the destructive nature of the pathogen and importance of the flower crop, the present investigation was initiated on important aspects of disease and the pathogen with the following objectives:

1. To conduct a survey for leaf spot severity in major marigold growing areas of North Karnataka.

2. Etiology, cultural and physiological studies of isolated pathogen.

3. *In vitro* and *in vivo* evaluation of fungicides, bio-agents and botanicals for the management of leaf spot disease.

2. REVIEW OF LITERATURE

Marigold (*Tagetes* spp.) is an important commercial flower crop grown in India. Alternaria leaf spot caused by *Alternaria alternata* (Fr.) Keissler is one of the major foliar disease occurring in all parts of the world, wherever marigold is grown. The studies with respect to survey, cultural and physiological and its management are taken into consideration while reviewing the literature. Accordingly, the literature pertaining to the above aspects is presented here.

2.1 Historical background

Shome and Mustafee (1966) for the first time reported the disease from West Bengal. They observed the infection of *Alternaria* species during 1964 on African marigold at floriculture, University of Kalyani and they identified the pathogen as *Alternaria tagetica* on marigold as a new species.

Yu and Lee (1989) reported a leaf, stem and flower blight of marigold (*Tagetes* spp.) caused by *Alternaria tagetica* for the first time in Korea 1987. Li *et al.* (2014) also reported for the first time that leaf spot disease of *T. erecta* was caused by *Alternaria alternata* during 2012 and 2013 in the Beijing district of china. Aktar and Shamsi (2014) for the first time reported that *Alternaria alternata* was found to be pathogenic on *T. erecta* and *T. patula* from Bangladesh.

2.2 Survey for leaf spot severity in major marigold growing areas of North Karnataka

Dhiman and Arora (1990) observed leaf spot and flower blight of *T. erecta* caused by *Alternaria tagetica* in Punjab. Average intensity and incidence were recorded as 51 and 83 %, respectively, in fields around Ludhiana. The severity of the disease significantly decreased the seed yield, viability, and damaged flower quality. On an average, infection reduced the seed weight and germination by 28 and 54 %, respectively. Seeds from diseased flowers produced 2-5 % diseased seedlings.

Survey was carried out at 10 villages of Gwalior district to appraise the intensity of Alternaria leaf spot and flower blight (*A. tagetica*) on the marigold crop. The per cent

disease intensity of leaf spot in surveyed localities of Gwalior district was 25.2 % to 35.83 %. The maximum incidence was found in Bhidole, Sighora, Talpura, and Sanjhana. The minimum disease intensity was found in Patpura, Panjabipura, and Bhawandpura. The incidence of flower blight was highest in Bhidole followed by Bhawandpura and Talpura. The minimum intensity of flower blight was found in Panjabipura followed by Udaypur and Baghpura. On the basis of weather conditions *i.e.* heavy rainfall and high relative humidity at flowering stages of the marigold crop, the disease incidence was highest during September to October (Patidar, 2000).

Akoijam (2007) conducted a survey of both African and French marigold growing areas in Solan and Sirmour districts of Himachal Pradesh during rainy season of 2006 and 2007. The average severity of Alternaria leaf spot in African marigold was highest at Nauni (79.30 %) and Thanedhar (79.20 %), followed by Churdhar and Shilabhat of Sirmour district with 75.07 and 75.93 per cent infection. The least leaf spot severity (63.93 %) was recorded at Kottaband in Sirmour district, whereas minimum (41.40 %) flower blight incidence was recorded at Mahog in Solan district.

Arunkumar (2008) carried out the survey during kharif/rabi 2007 in the districts of Dharwad, Gadag, Haveri, and Koppal to get precise information on the incidence of *Alternaria* leaf blight on chrysanthemum and found that the highest incidence was observed in Kurabagatti village (78.42 %) fallowed by Rayanala village of Dharwad district (70.00 %). Haveri, Gadag and Koppal districts recorded a leaf blight incidence of 49.70, 46.33 and 40.93 per cent respectively.

Roving survey on the incidence of Alternaria leaf spot of marigold was conducted in four districts of Himachal Pradesh *viz.*, Shimla, Solan, Sirmour and Mandi during the years 2014 and 2015 cropping season in the months of August to October. The data revealed that the disease index on the leaves of marigold varied from 63.2 to 83.2 per cent. The disease incidence on flowers in total varied from 56 to 84 per cent during 2014 and 2015. The highest per cent disease incidence on leaves was recorded in Nauni (82.0 %) followed by Narag (81.2 %) and Dilman (79.2 %). While Shimla district had the least disease index (64.4 %) on leaves. The disease incidence on flowers was highest at Nauni (83.0 %) followed by Narag and Dilman of Sirmour district with 81.0

per cent. The lowest disease incidence (58.0 %) on flowers was recorded at Shimla (Negi, 2016).

2.3 Symptomatology

A. zinniae, *A. tagetica* and *A. alternata* are the three species of Alternaria infecting marigold. Symptoms caused by *Alternaria zinniae* appear as dark brown necrotic spots developed on leaves, leaf stalks and flowers in the upper part of the plant (Edward, 1957). Shome and Mustafee (1966) reported that as the severity of the disease increases, spots enlarge and coalesce to cover most of the leaf lamina and also infect flower heads.

Sen (1996) observed the leaf spot and flower blight of marigold in the year 1995 as severe spotting on leaves, stems and inflorescence including sepals and petals giving a blighted appearance to the entire plant. On the basis of pathogenicity test the fungus was identified as *A. zinniae.*

Verma and Sharma (1999) and Patil (2003) observed leaf spot of marigold first on lower older leaves and progressing upward. *A. alternata* caused circular, brown lesions that later enlarged, coalesced and turned dark brown to black. Severely infected plants became black, appeared scorched and eventually died. On blooming, the inflorescence axis and flower heads were attacked severely and turned dark brown to black.

In case of *Alternaria tagetica*, the disease occurred as minute brown to dark brown, circular to oblong, necrotic spots on the older leaflets towards their margin. The adjacent spots subsequently consolidated with one another to form large irregular patches. Concentric rings were also observed in some enlarged old spots. On blooming, the inflorescence axis and flower heads are attacked. The severely attacked flower buds failed to open and dried, resulting in breaking and drying of unopened flower buds (Uke, 2011).

Symptoms produced by *Alternaria alternata* on the affected plants had small, brown, necrotic spots on most of the foliage. As the disease severity increases spots

gradually enlarge, become irregular in shape, or remained circular and with concentric rings or zones. The leaves wither, dry and shed from the plants (Li, 2014).

2.4 Pathogenicity

Inoculated leaves of turmeric plants with a spore suspension of the pathogen produced symptoms in susceptible plants after 6 days of inoculation. Similarly, *A. solani* was reported pathogenic to seedling, mature fruits and leaves of brinjal in inoculation test (Ranasingh *et al.*, 2006 and Thippeswamy *et al.*, 2006). Wenneker *et al.* (2006) have proven the pathogenicity of *A. alternata* on the detached dormant flower bud of pear and showed pronounced death after 7 days on susceptible plants.

Kashyap and Dhiman (2009) conducted pathogenicity test on 45 days old potted cauliflower seedlings by spraying the conidial spore suspension of $5x10^5$ conidia/ml *Alternaria brassicola* and covered the plant with polythene bag for 48 hours for observation of symptoms.

Sharma *et al.* (2012) proved the pathogenicity of *Alternaria alternata* causing blight in pigeon pea by inoculating seedlings with conidial (5×10^5 conidia/ml) suspension of 7 days old culture and covered with a polythene bag and incubated at 28 ± 1 °C. After ten days, symptoms on leaves were developed, which were identical to the observed symptoms in the field.

The pathogenicity study conducted by Aktar and Shamsi (2014) showed that different isolates of pathogen varied in their ability to cause leaf spot and flower blight on a marigold. They also tested the pathogenicity of leaf spot and flower blight disease by spraying water suspension of test fungus (*Alternaria alternata*) at a 10^4 ml concentration. The pathogen, *Alternaria alternata* showed the symptoms of disease on all the inoculated plants of *Tagetes* spp.

2.5 Morphological and Molecular identification of Alternaria leaf spot of marigold

Keissler (1912) observed that the colonies of *A. alternata* were usually black or olivaceous black and sometimes grey. Conidiophores produced singly or in small groups, simple or branched, straight or flexuous, sometimes geniculate, pale to mid

olivaceous or golden brown, smooth, up to 50 µm long, 3-6 µm thick, with one or several conidial scars. Conidia formed in long often branched chains, obclavate, pyriform, ovoid or ellipsoidal often with short conical or cylindrical beak sometimes up to one but not more than one third the length of the conidium, pale to mid golden brown, smooth or verruculose with upto eight transverse and usually several longitudinal or oblique septa. Overall length 20-63 µm, 9-18 µm thick in the broadest part, beak pale, 2-5 µm thick.

Karlatti and Hiremath (1989) found that the pathogenic hyphal culture of *Alternaria zinniae* from *Tagetes erecta* formed good, dark brownish to black mycelial growth with numerous transverse and longitudinal septation.

Pandey and Vishvakarma (1999) observed the mycelial growth characters of *A. alternata* causing leaf spot of brinjal as circular, profuse cottony and wooly, olivaceous black, with white periphery on potato dextrose agar medium.

Prasanna kumar (2001) reported that conidiophores of *Alternaria alternata* were simple, rather short or elongate, typically bearing a simple or branched chain of conidia; conidia were dark, typically with both cross and longitudinal septa; variously shaped, obclavate to elliptical or ovoid, frequently borne acropetally in long chains, less often borne singly.

Mirkova and Konstantinova (2003) observed that the conidia of *A. alternata* isolated from gerbera were catenated in long, sometimes branched chains of 5-12 spores, variable in size and shape, usually ovoid to ellipsoid or obclavate and with a usually pale long oval conidia, pale brown to brown, with 3-6 transverse and 0-2 longitudinal or oblique septa and measured 25-35x5-10 µm.

Patil (2003) noted the morphological characters of *A. alternata* isolated from marigold. The mycelium was irregular at acute angle, the hyphae were smooth, septate, profusely branched, light to dark brown in colour, 3.7-5.5 µm in diameter, conidiophores were erect, grouped, unbranched, brown to dark brown, septate (1-6 septa), 37.2-124.0 µm x 6.2-9.3 µm in size, muriform conidia with 3-9 transverse and 1-4 longitudinal septa in younger and 8-14 transverse and 3-6 longitudinal in older ones,

deep brown in colour, showed a wide central part -tapering at both the ends and measuring 186-217 µm x 8.6-31.0 µm in size.

Guo *et al.* (2004) sequenced the three unknown isolates of Alternaria species. The result indicated that this sequence had highly similarity with reference to ITS sequences of *A. alternata* in GenBank, particularly identical with some reference ITS sequences, e.g. accession nos. AF455537, AF455441, AF397233, AF397242, and AY160211.

Xie *et al.* (2012) identified and separated three pathogenic fungi of tomato by using trans conjugate method, morphological characteristics and internal transcribed spacers ribosomal DNA (ITS rDNA) sequence analysis. The results of morphological observation and molecular detection of three fungi revealed that FQ1 belong to *Alternaria alternata*. FQ2 belong to *Cladosporium* sp. FQ3 belongs to *Fusarium* sp.

Li *et al*. (2014) amplified the rDNA of the internal transcribed spacer regions 1 and 2 and the 5.8S gene in seven isolates of leaf spot of *T. erecta* using primers ITS1 (5′ TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGA TATGC-3′). The nucleotide sequence was the same as isolate No. 7, which was deposited in Gen Bank (Accession No. KF307207). A BLAST search revealed that 97 % identity with the strain *Alternaria alternata* GNU-F10 (KC752593). Seven isolates were further confirmed as *A. alternata* by PCR identification performed by specific primers of *A. alternata*.

Dutta *et al.* (2015) isolated the fungal culture from severe blight and dieback disease on water hyacinth. To confirm the species of the fungi, the isolate was subjected to molecular characterization by amplification of 18S RNA gene fragment from genomic DNA using 18S gene universal primers. The fungus was detected as *Alternaria japonica* Yoshii by Gen Bank database comparisons and phylogenetic analysis during sequencing.

Chethana *et al.* (2018) carried out the molecular characterization of geographical isolates based on the sequence of the ITS region of rDNA. Sequencing and blasting of ITS rDNA of isolates reported 100 % matching of five different species of Alternaria

viz., *A. porri*, *A. alternata*, *A. tenuissima*, *A. palandui* and *A. brassicicola*. The differentiation between the species and among the isolates by Cluster analysis of ITS rDNA sequences was not reliable, therefore suggested that region of ribosomal DNA gene is inappropriate for taxonomic resolution of Alternaria species infecting onion.

Luo *et al.* (2018) identified a single spore isolate of leaf spot disease on *Chrysanthemum coronarium* based on morphology and sequence analysis using four regions (rDNA ITS, GAPDH, EF-1a, and RPB2). The results indicated that the fungus is *Alternaria argyranthemi* and during pathogenicity tests revealed that the species could cause severe leaf spot and blight disease on the host. This is the first report of leaf spot disease on *C. coronarium* caused by *A. argyranthemi* in the world, which is also a new record of Alternaria species in China.

Prathima *et al.* (2018) conducted a molecular characterization of the isolate causing Alternaria leaf blight of marigold at the genus level utilizing the ITS primers resulted in amplification of a 528 bp fragment and sequenced rDNA-ITS region. Use of Alternaria species specific primers AaF and AaR assured that this isolate as *Alternaria alternata* at the species level. Phylogenetic tree analysis was hoisted out to know the diversification of the isolate and the results showed that *A. alternata* is diverse from the other Alternaria isolates reported earlier from different host plants.

2.6 Cultural and physiological studies on isolated pathogen

De Tempe (1986) recognized that deep seed infection are responsible for pre-emergence rot, while surface seated one caused disease on developing plants. The infected seeds with *Alternaria* spp. produced seed rotting and 100 per cent seedling fatality of the Cosmos plant (Srivastava and Gupta, 1981).

Srivastava (1986) also found the fungus in the seed coat and embryo of the *Zinnia elegans.* Meredith (1996) found that sporulation of *A. porri* occurred at night under relative humidity and dew formation.

Yang and Wu (1992) revealed that the longevity of the pathogens (*Alternaria zinnia*) is influenced by two important factors like temperature and soil moisture. While

the temperature of 40 °C desiccated the spores. A wide optimum temperature range of 21-30 °C favored spore germination and symptoms production and high relative humidity favored the appearance of typical blotch in onion (Bocks, 1964).

2.6.1 Effect of solid and liquid media on growth of isolated pathogen

Raju and Mehta (1982) reported that potato dextrose medium was best for the growth of the fungus and sporulation occurred in the absence of the light after the growth for 96 hours in darkness followed by exposure to sunlight for 2 hours.

Kantwa *et al.* (2006) reported that maximum growth and sporulation of *Alternaria alternata* was recorded on potato dextrose broth (54.75 mg and 13.75 ^{x} 10^{6}/ ml) followed by Richard's, oatmeal, Czapek's, corn meal and Asthana broth.

Hubballi *et al.* (2010) observed all the fifteen isolates of *A. alternata* on eight different media. Among them, host leaf extract medium supported significantly the maximum growth followed by potato dextrose agar (PDA). Minimum mycelial growth was seen on water agar.

Ramjegathesh and Ebenezar (2012) observed that all twelve media supported separately the growth of the ten isolates of *A. alternata.* The maximum mycelia growth of the pathogen was found in host leaf extract agar medium (9.00 cm) pursued by Czapek dox agar (9.00 cm), potato dextrose agar (8.14 cm) and carrot agar medium (7.94 cm), while it was less in glucose phosphate agar (5.14 cm) and too less in water agar medium (0.92 cm).

Nagrale *et al.* (2013) reported that in non-synthetic media, Oat meal agar and PDA showed excellent mycelial growth and conidial production of *Alternaria alternata* causing blight of gerbera than in synthetic media *viz.*, Leonions's agar, Glucose-peptone agar and Sabourand's agar.

Gholve *et al.* (2015) reported that all eight culture media tested encouraged better growth of *Alternaria carthami*. However, Potato dextrose agar gave the highest growth (90.00 mm). The second and third best culture media found were Potato malt agar (84.16 mm) and Yeast manitol agar (73.33 mm). Remaining culture

media recorded a good amount of mycelial growth between 41.66 mm (Yeast extract agar) to 69.16 mm (Malt extract agar).

Koley and Mahapatra (2015) studied the growth of the *Alternaria solani* culture in twelve different liquid and solid media. Potato dextrose agar and oat meal agar among solid media and Richard's broth and Sabouraud's broth among liquid media showed maximum growth.

Devappa and Thejakumar (2016) revealed that the *A. alternata* showed maximum mycelia growth on Richard's agar (90.00 mm) followed by oat meal agar (89.00 mm), Asthana and Hawker's medium (85.33 mm) and Potato dextrose agar (85.00 mm). The maximum sporulation was observed in Potato dextrose agar followed by Richards's agar, Oat meal agar and Asthana and Hawker's medium.

Reddy *et al.* (2019) reported the maximum radial mycelia growth of the *A. alternata* (90 mm) in PDA with excellent sporulation (++++) and poor sporulation was observed in Czapek's dox agar.

2.6.2 Effect of temperature on growth of *A. alternata*

Green and Bailey (2000) observed equal conidial germination of *Alternaria cirsinoxia* at 10, 15, 20, 25, or 30 °C. However, formation of appressoria and leaf penetration occurred maximum at all 20 to 25 °C and 20 °C, respectively.

Hubballi (2010) reported that all the fifteen isolates of *Alternaria alternata* causing leaf blight of noni grew well at a temperature of 30 °C (89.45 mm) followed by 25 °C (86.11 mm) and 35 °C (70.27 mm). The lowest growth was observed at 5°C (9.70 mm).

Taware (2014) observed effects of different temperature for better growth of *Alternaria carthami*, at the highest mean mycelial growth (85.66 mm) was at 30 °C followed by 25 °C (83.83 mm) and 20 °C (66.33 mm).

Chohan (2015) reported that among different isolates of *Alternaria solani* subjected to different temperature range, the significant growth (7.56 cm) was observed

at 25 °C followed by 35 °C, 20 °C and 30 °C with 7.28, 5.36 and 5.00 cm mycelial growth respectively.

Sinha and Alam (2017) observed maximum radial growth of mycelium and the better sporulation of *Alternaria solani* of tomato at 28 °C (82.76 mm) followed by 32 °C (81.18 mm) in solid media. The dry mycelial weight at 28 °C was 1686 mg/l of culture, while it was 1570 mg at 32 °C.

Gunda *et al.* (2018) studied the growth of *Alternaria brassicicola* at different temperatures *i.e.*, 15, 20, 25, 30 and 35 °C and found that the growth was maximum at 25 °C (9 cm) followed by 20 °C (6.3 cm). There was complete inhibition of growth at 35 °C. Optimum temperature for the growth of pathogen was observed as 25 °C and further increase or decrease in temperature inhibited the growth.

2.6.3 Effect of pH on growth of *A. alternata*

Akoijam (2007) reported that pH levels between 5.5 and 6.0 supported the maximum (226.27 mg and 278.87 mg) growth, followed by 5.0 (200.03 mg) of *Alternaria zinniae* causing leaf spot and flower blight of marigold. As pH levels were increased above 6.5, there was an abrupt decrease in dry mycelial weight. Minimum dry mycelium weight (121.20 mg) of the test fungus was achieved at pH 7.5.

Hubballi (2010) observed that the mean mycelial growth was maximum at pH 6.5 with 87.50 mm followed by pH 6.0 (84.60 mm) and pH 7.0 (70.00 mm) and least was observed at pH 4.0 (28.00 mm).

Odenapur (2011) reported that maximum growth (403.33 mg) was recorded at pH 6 followed by pH 5 (380 mg), pH 4 (293.33 mg), pH 7 (235.67) and least growth of *Alternaria alternata* of leaf blight of chrysanthemum was obtained (204.00 mg) at pH 8.

Taware (2014) revealed that *Alternaria carthami* at pH 6.5 recorded maximum mean mycelial growth (85.83 mm) with excellent sporulation, followed by at pH 6 (82.00 mm) and pH 7 (70.33 mm) with good sporulation.

Rout *et al.* (2015) reported the effect of different level of pH on *Alternaria alternata* causing collar rot of marigold, where it grows best in acidic medium with 4 pH.

Choudhary (2017) reported that *Alternaria alternata* causing leaf blight of Isabgol preferred pH 6.5 by producing the maximum dry mycelial weight of 845 mg followed by pH 6.0 (820 mg). The dry mycelial weight was least at pH 9, which recorded 290 mg. The pH below six and above 6.5 was discerned to be inhibitory to the growth.

Kumar *et al*. (2017) found out the optimal pH range for *Alternaria sesami* mycelial growth from 6.0 to 6.5. Good sporulation was observed at pH 7.0 to 7.5 and found poor sporulation at pH 4.5.

Sinha and Alam (2017) studied the effect of pH on both solid and liquid media and observed maximum radial growth of a fungus at pH 7.0 (86.28 mm) followed by pH 7.5 with 85.24mm. In liquid media, the highest dry mycelial weight was observed at 7.0 pH (1620 mg) followed by 7.5 pH (1540 mg).

Gunda *et al***.** (2018) observed that the radial growth of *Alternaria brassicicola* was maximum at pH 5.5 (9 cm) and 6 (9 cm) followed by pH 6.5 (8.6 cm) and pH 5 (8.4 cm) and the least growth of the pathogen was recorded at pH 7 (7.6 mm).

2.7 *In vitro* and *in vivo* evaluation of fungicides, botanicals and bioagents for the management of leaf spot disease

2.7.1 *In vitro* evaluation of fungicides against *A. alternata*

Murthy and Shenoi (2001) reported that difenconazole (Score), propicanazole (Tilt) and mancozeb (Indofil M-45) were potent in inhibiting the mycelial growth even at 100 ppm concentration against *A. alternata*. Arun Kumar (2008) also reported that propiconazole and hexaconazole were best at all concentrations (0.1 %, 0.2 % and 0.3 %) which completely inhibited the mycelial growth.

Patil (2003) reported that propiconazole (Tilt 25 EC at 250, 500, and 1000 ppm), difenconazole (Score 25 EC at 500 and 1000ppm) gave cent per cent inhibition of the mycelial growth and spore formation of *A. alternata* isolated from marigold. Patel (2003) also recorded propiconazole totally inhibiting the mycelia growth of the *A. alternata* of greengram at 250, 500 and 1000 ppm concentrations. Propineb, ziram and thiophanate methyl were also observed effective especially at higher concentrations. Mixed fungicides *viz.*, metalaxyl + mancozeb and carbendazim + mancozeb proved moderately effective while mancozeb and copper oxychloride were less effective. Carbendazim and chlorothalonil were the poorest in their effectiveness.

Prasad and Naik (2003) reported that the copper oxychloride was one among the non-systemic fungicides effective in inhibiting the growth of *A. solani* and *A. alternata.* Mantecon (2007) reported that the most effective control of *A. solani* was achieved by copper oxychloride (64.7 %) followed by mancozeb (61.7 %).

Jash *et al.* (2004) reported that Mancozeb 75 %WP (Indofil M-45), Metalaxyl 8 % + Mancozeb 64 % WP (Tata master) and Carbendazim 12 % + Mancozeb 63 % WP (Companion) @ 200 ppm each totally inhibited the mycelial growth of the *Alternaria zinniae* under *in vitro* condition over untreated control whereas Bordeaux mixture, Carbendazim 50 WP and Copper Oxychloride 50 % WP at 500 ppm each exhibited inhibition of mycelial growth of the pathogen.

Odenapur (2011) tested five non-systemic fungicides with one combi product at three concentrations *i.e.* 0.05 %, 0.1 %, and 0.2 % against *A. alternata*. The maximum inhibition of mycelial growth (93.08 %) irrespective of the concentration was recorded in Avatar (Hexaconazole 4 %+ Zineb 68 %) followed by Mancozeb (90.82 %), Zineb (82.37 %), Chlorolthalonil (81.10 %) which were significantly superior over the other treatments. Captan reported the least inhibition (73.10 %) of mycelial growth.

Gholve *et al.* (2012) evaluated six fungicides *viz.*, Mancozeb 75 WP; Carbendazim 50 WP, Copper oxychloride 50 WP, Captan 50 WP, Thiram 75 WP, Chlorothalonil 75 WP under *in vitro* condition at 500, 1000 and 1500 ppm against *A. macrospora*. Thiram was found most effective and recorded highest mean mycelial

inhibition (90.42 %) followed by Captan (82.04 %), Mancozeb (79.88 %), Carbendazim (77.50 %), Chlorothalonil (74.52 %) and Copper oxychloride (71.75 %).

Kumar *et al.* (2013) reported that among 12 fungicides evaluated against Alternaria leaf spot disease of chilli only five fungicides *viz.*, Carbendazim, Mancozeb, Chlorothalonil, Vitavax and Thiram proved to be effective fungicides in the inhibition of mycelia growth. The remaining fungicides in order of their decreasing inhibitory effect against the pathogen were Zineb, Captafol, Ziram, Captan, Copper oxychloride, Iprobenfos and Thiophanate methyl.

Roopa *et al.* (2014) evaluated twelve fungicides against *Alternaria solani* causing early blight of tomato. The results revealed that contact fungicide mancozeb @ 0.2 %, a systemic fungicide, hexaconazole @ 0.1 % and the combi fungicide Hexazconazole 4 % + Zineb 68 % @ 0.2 % marked the maximum inhibition of 87.21, 88.88, 88.88 per cent mycelial growth respectively.

Waghe *et al*. (2015) evaluated six fungicides at 500, 1000, 2000 and 2500 ppm against *Alternaria helianthi* causing leaf spot of sunflower. Among the fungicides tested the maximum inhibition was observed in Carbendazim 12 % + Mancozeb 63 % WP at 2000 ppm (90.36 %), followed by Mancozeb at 2500 ppm (88.88 %). The least resistance was observed in Azoxystrobin at 500 ppm (72.96 %).

Wagh *et al.* (2017) evaluated six systemic fungicides at 500, 1000 and 1500 ppm concentration and seven contact / combi fungicides at 1000, 2000 and 2500 ppm concentrations against *A. carthami*. The study revealed that systemic fungicides were most effective compared to non-systemic against the test pathogen. Among systemic fungicides, average inhibition of mycelial growth was observed in Hexaconazole (100 %), followed by Propiconazole (94.07 %) and Penconazole (94.75 %); Among non-systemic and combi- fungicides, significantly higher average mycelial growth inhibition was observed in Carbendazim 12 WP + Mancozeb 63 WP (85.80 %), followed by Mancozeb (82.59 %) and Copper-oxychloride (76.65 %).

Prasad *et al.* (2018) examined the result of eleven fungicides against *A. macrospora*. Among them mancozeb, carbendazim, hexaconazole, propiconazole and carbendazim + mancozeb (each at the recommended dose, 500 ppm below their

recommended dose and 500 ppm above their recommended dose) inhibited (100 %) the growth of *A. macrospora* followed by captan + hexaconazole (89.74 %), thiram and captan (78.89 %), Azoxystrobin exhibited the least inhibition (56.81 %) compared to an untreated check.

2.7.2 *In vitro* evaluation of botanicals against *A. alternata*

Meena and Mariappan (1993) evaluated the leaf extracts of *Mentha arvensis, Catharanthus roseus, Lantana camara, Pongamia pinnata, Vitex negundo* and *Nerium odorum* (*Nerium olender*), *Azadirachta indica,* and flower extracts of *Catharanthus* against seed borne mycoflora of sorghum including *Alternaria tenuis, Alternaria alternata, Aspergillus flavus, Curvularia lunata* (*Cochlibolus lunatus*) and *Fusarium moniliforme* (*Gibbrella fusikuroi*). The more effective result was observed in the extracts of Neem, *Catharanthus roseus,* and *Lantana camara* as compared to all other plant extracts.

Apet *et al.* (2014) recorded the highest inhibition of mycelial growth in *Allium sativum* (74.45 %), followed by *Curcuma longa* (63.99 %), *Datura metel* (53.06 %), *Calatropis gigantica* (48.99 %) and *Parthenium hysterophorus* (48.90 %) against *Alternaria alternata* causing leaf spot of gerbera**.**

Leaf extracts of six plants *viz*, *Azadirachta indica*, *Jatropa curcas*, *Datura strumarium*, *Moringa oleifera*, *Calotropis gigantea,* and *Morus alba* each at 50 per cent concentration were tested against *Alternaria alternata* causing leaf spot of *Aloe vera* and all the leaf extracts inhibited the mycelial growth of the pathogen. However, Jatropa leaf extract showed maximum mycelial growth inhibition *(*62.9 %) pursued by *D. strumarium leaf* extract (55.6 %), *A. indica* extract (51.9 %), *M. oleifera (*46.9 %), *C. gigantea* extract (23.45 %) and least was observed in *M. Alba* (13.6 %) (Regmi *et al*., 2014).

Mahantesh *et al*. (2017) conducted an experiment on *in vitro* evaluation of botanicals against *Alternaria solani*. The results revealed that Pongamia leaf extract showed maximum inhibition of mycelia growth (54.76 %) at 10 % followed Neem leaf extract (48.27 %), Lantana leaf extract (47.42 %). Significantly, Pongamia leaf extract showed 46.25 % of inhibition at 5 % followed by Lantana (43.58 %) and Neem leaf

extract (42.92 %). Whereas, water hyacinth recorded the least inhibition of mycelial growth.

Lingaraj *et al*. (2018) evaluated six botanicals against *Alternaria alternata* at two concentrations (5 % and 10 %) by poisoned food technique. All the treatments were found to be significant. Prosopis (49.05 %) was superior over all other plant extracts. The next best treatment was parthenium (22.53 %), which was on par with NSKE (20.83 %). Least inhibition was recorded in clerodendron (11.05 %).

Yedida and singh (2018) conducted *in vitro* evaluation of various botanicals against *Alternaria cyamopsidis.* The results revealed that *Azadirachta indica* and Eucalyptus were superior over all other treatments followed by *Allium cepa, Zingiber officinalis*, Parthenium and Datura. The least mycelial growth was observed in *Lantena camera*.

2.7.3 *In vitro* evaluation of Bioagents against *A. alternata*

Ganie *et al*. (2013) reported that *T. harzianum* caused maximum mycelial growth inhibition of 71.85 % in dual culture, followed by *T. viride* (65.93 %) in *A. solani.*

Rahman *et al.* (2015) tested the antagonistic effects of four biocontrol agents *viz., Trichoderma viride, T. koningii, T. harzianum, Bacillus sp*. on mycelia growth of *Alternaria porri* and maximum reduction in colony growth of *A. porri* was observed due to *T. viride* and pursued by *T. harzianum.* The least effective was *Bacillus sp.*

Waghe *et al*. (2015) conducted an experiment on *in vitro* evaluation of biocontrol agents against *A. helianthi* with three fungal (*Trichoderma viride, T. harzianum* and *T. hamatum*) and one bacterial (*Pseudomanas fluorescens*) bioagents by applying dual culture technique. The results revealed that all the bioagents with inhibition of mycelial growth of *A. helianthi* exhibited the fungistatic activity. Among the four bioagents tested, least mycelial growth (25.00 mm) was observed in *T. harzianum* with a maximum inhibition of mycelial growth (72.22 %) of the test pathogen, over untreated control followed by *T. viride* (70.27 %) and *T. hamatum*

(51.66 %). *Pseudomonas fluorescences* was found to be comparatively least effective with 46.25 mm linear mycelial growth and 48.60 % mycelial inhibition.

Wagh *et al.* (2017) revealed that *T. viride* recorded the maximum mycelial inhibition (87.04 %) of *Alternaria carthami* causing leaf spot / blight of Safflower under *in vitro* condition followed by *T. harzianum* (82.59 %) and *T. koningii* (78.89 %).

Hariprasad *et al*. (2018) proclaimed that maximum inhibition (100 %) was recorded in the fungal biocontrol agents, *T. harzianum* (NBAIR), followed by *T. viride* (81.38 %) whereas the bacterial bioagent, *Bacillus amyloliquefaciens* (P-42) showed hardly 77.40 % inhibition of mycelia growth. The results revealed that fungal antagonists are better than bacterial antagonists.

The antagonistic effect of five bioagents *viz.*, *T. viride*, *T. harzianum*, *B. subtilis*, *P. fluorescens* and *Myrothecium inundatum* were evaluated against *A. brassicicola* under *in vitro* conditions. The results revealed that the highest percentage of inhibition of mycelial growth was recorded with *T. viride* (82.20) which is on par with *T. harzianum* (80.00), followed by *B. subtilis* (64.40). The least percentage of inhibition was recorded with *P. fluorescens* (14.40) (Gunda *et al*., 2018).

Valvi *et al.* (2019) evaluated three bioagents *viz.*, *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* against *Alternaria brassicae* (Berk.) Sacc. causing *Alternaria* leaf spot of cauliflower. The study revealed that *T. viride* recorded maximum inhibition of *A. brassicae* when placed at both periphery (72.46 %) and at centre (68.48 %) over control. This was followed by *T. harzianum* which showed 67.57 and 62.64 per cent inhibition of the test pathogen. The least inhibition was observed in *Pseudomonas fluorescens* at both the condition 61.03 per cent when streaked centrally and 55.33 per cent when streaked on two sides of test pathogen.

2.7.4 *In vivo* evaluation of fungicides, botanicals, bioagents against *A. alternata*

Dubey (2001) tested five plant extracts against leaf spot and flower blight of marigold (*Alternaria tagetica*). Among the plant extracts / products the neem leaf extract at 10 % was found most effective under field condition and controlled the

disease by 47.3 % and 31.83 % on leaf and flower, respectively and increased the flower yield by 48.7 %.

Patil (2003) found propiconazole while Patel (2003) found propiconazole and ziram as the most effective fungicides in controlling marigold and green gram leaf spot (*A. alternata*) diseases, respectively.

Kumar *et al.* (2011) conducted a field evaluation of fungicides, botanicals, and bio-agents against Alternaria leaf blight of chrysanthemum. Hexaconazole (0.1 %) showed very less per cent disease index (4.49) followed by Chlorothalonil (0.2 %) and Mancozeb (0.2 %). Hexaconazole recorded the highest yield (76.25 q/ha) and incremental benefit: cost ratio of 7.16. Hence, it can be recommended to the farmers as an economical disease management practice.

Archana and Jamadar (2012) conducted a field trial on efficacy of systemic and non-systemic fungicides against *A. alternata* on pomegranate and found that propiconazole (PDI, 10.6) thiophanate methyl (PDI, 14.7) and copper oxychloride (PDI, 23.4) were more efficient among the tested fungicides.

Gohel *et al.* (2012) conducted an *in vivo* evaluation of fungicides against alternaria leaf spot of chilli. The results revealed that maximum disease control was observed in the plot sprayed with propiconazole (83.46 %) which was on par with difenconazole (78.39 %) followed by mancozeb (68.58 %). Chlorothalonil and carbendazim + mancozeb were found to be least effective against *Alternaria alternarta.*

Nagrale *et al.* (2012) reported that Bordeaux mixture (0.6 %), tricyclazole (0.1 %) and iprodione + carbendazim (0.1 %) fungicides as preventive sprays were found to be more effective with 95.85 %, 96.59 % and 95.88 % disease control respectively, under polyhouse condition against Alternaria blight of gerbera caused by (*A. alternata)*. The curative sprays with tricyclazole (0.1 %), iprodione + carbendazim and Bordeaux mixture (0.6 %) showed the least disease severity with the maximum disease control of 95.88, 94.83 and 95.85 per cent respectively. The pursued fungicide treatments in order of supremacy were difenoconazole (0.1 %), propiconazole (0.1 %) and hexaconazole (0.1 %) which recorded disease severity of 2.08, 2.92 and 3.08 per cent respectively with a disease control of 93.40, 90.96 and 90.46 per cent respectively. Hence, concluded

that preventive fungicidal sprays were significant over curative fungicidal sprays for the management of *Alternaria alternata* blight of gerbera under polyhouse condition.

Nashwa *et al*. (2013) evaluated six plant extracts at 5 and 10 % concentrations. *O. basilicum, A. indica, E. chamadulonsis, D. stramonium, N. oleander,* and *A. sativum*, significantly reduced the early blight disease. The most effective treatments with plant extracts were *A. sativum* followed by *D. stramonium*. The least reduction of disease index was achieved by *O. basilicum* at 1 % concentration (35.2 %). Other treatments were moderately effective against *Alternaria solani.*

Bhat *et al.* (2017) evaluated the different fungicides under natural epiphytotic condition and found that all the tested fungicides proved significantly superior over check in controlling the leaf blight of gerbera (*Gerbera jamesonii* Hook). Fulusilazole 40 EC at 200 ppm was the most effective fungicide with 88.28 per cent disease control followed by chlorothalonil 75 WP at 3000 ppm with 82.64 per cent disease control.

Kumar *et al.* (2017) evaluated four botanicals and one bioagent against *Alternaria carthami* leaf blight of safflower during Rabi season 2016-2017. The minimum disease intensity was observed in Datura (30.97 %) followed by Lantana (34.18 %), Neem (37.30 %), Eucalyptus (42.45 %), *T. harzianum* (46.36 %) as compared to the treated control SAAF (22.82 %) and untreated control (56.73 %).

An experiment was conducted to test the efficacy of two plant extracts, two bio-agents and two fungicides under *in-vivo* against *Alternaria brassicae* causing leaf spot of cauliflower. Propiconazole at 0.2 % (21.30) exhibited minimum per cent disease intensity followed by Mancozeb at 0.2 % (23.15), Eucalyptus at 5 % (24.29), *Lantana camera* at 10 % (25.19), *T. harzianum* at 2.0 % (25.78), *P. fluorescence* at 2.0 % (27.04) as compared to untreated control (28.98). The plants treated with propiconazole recorded highest yield (70.06 t/ha) as compared to untreated control which recorded only 31.58 t/ ha (Sailaja *et al*., 2017).

Shindhe *et al.* (2018) reported that hexaconazole (0.1 %) was found effective in reducing the per cent disease index (32.15) on leaves and (33.76) on a flower in all three sprays and getting higher yields (6.96 t/ha) in marigold followed by mancozeb (0.2 %) with PDI of (34.53) on leaves and (35.45) on the flower with a yield of (6.81 t/ha) as

compared to control with (85.02 and 86.11 PDI) on leaves and flower respectively with the yield of (4.26 t/ha). *Trichoderma harzianum* (5 g/l) was effective in minimizing the per cent disease index (17.07) on leaves and (17.37) on flowers and recorded yield (5.26 t/ha). Singh and Singh (2006) also obtained highest disease control and flower yield in marigold with the application of difenoconazole and penconazole.

3. MATERIAL AND METHODS

Studies on Alternaria leaf spot of marigold were conducted in the Department of Plant Pathology, College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka during 2018. The material and methodologies adopted during the course of study are elaborated below.

Glass ware used

In different experiments of *in vitro* studies, Borosil glassware was used comprising of Petri plates (90 mm diameter), test tubes (10 and 20 ml), microscopic slides (25 × 75 mm), Erlenmeyer flasks (100, 250 and 500 ml), beakers (500 and 1000 ml), measuring cylinders (10 ml, 50 ml and 1000 ml) and pipettes (1, 5 and 10 ml) were used during the course of investigations.

Sterilization

The sterilization of glass ware was done by autoclaving at 121 ^{o}C at 15 psi for 15- 20 minutes. Autoclaved glass ware was dried in hot air oven at 80 ^{o}C for 45 to 60 minutes. All *in vitro* experiments were carried out under aseptic conditions using laminar air flow. Inside the laminar air flow, spirit lamp flame was used for sterilizing inoculation needle and also for inoculation of the pathogen culture.

3.1 Survey and collection of diseased samples

Roving survey was carried out for recording the severity of leaf spot in major marigold growing areas of North Karnataka *viz*, Bagalkot, Koppal, Haveri and Belagavi. The disease samples were collected for isolation of the pathogen and cultural studies. The data on disease severity was recorded on randomly selected five plants/100 Sq mt at each location using 0-5 scale (Negi, 2016) and Per cent Disease Index (PDI) was calculated.

Scale (0-5) for recording observations

Grade/scale	Description
0	No visible symptoms
1	One or two spots or < 5 % leaf area affected
2	3 to 5 spots or < 10 % leaf area affected
3	11 to 25 % leaf area affected
4	25-50 % leaf area affected
5	>51 % leaf area affected

The Per cent Disease Index was calculated by using the formula given by wheeler (1969)

$$\text{Per cent Disease Index (\%)} = \frac{\text{Sum of all numerical ratings}}{\text{Number of observed leaflets} \times \text{maximum disease scale}} \times 100$$

3.2 Isolation and identification of causal organism

3.2.1 Isolation of the pathogen

Infected leaves of marigold showing typical symptoms of leaf spots were used for isolation of the fungus. The infected part of the plant was cut into small pieces such a way that each bit comprise of infected as well as healthy tissues. These bits were surface sterilized by dipping in 0.1 per cent mercuric chloride ($HgCl_2$) solution for 20-30 seconds and then washed thrice with sterilized distilled water to remove the traces of mercuric chloride and then aseptically transferred on sterilized Petri plates containing 20 ml Potato Dextrose Agar (PDA) medium and these Petri plates were incubated at 27 ± 2 ^{o}C temperatures. The fungal hyphae developing from the infected tissues were sub-cultured aseptically on PDA slants and pure culture thus obtained was maintained. This culture was examined microscopically for identification and was further purified by using single spore isolation technique. The single spore culture obtained was later maintained on PDA slants for further investigation.

3.2.2 Single spore isolation

Ten ml of two per cent sterilized water agar was poured into sterile petri plates and allowed to solidify. A dilute spore suspension was formulated in sterilized distilled water from a 15-day-old culture. One ml of such suspension was distributed evenly on agar plate. These plates were incubated at 27 ± 1 ^{o}C for 12 hr. Then, such plates were examined under a microscope to locate single isolated and germinated conidium and pointed with ink on the surface of the plates. The growing hyphal tip part was transferred to PDA slants with the help of a cork borer under aseptic conditions and incubated at 27 ± 1 ^{o}C. Such culture tubes were adopted for further studies.

3.2.3 Identification of the pathogen

The morphological characters of the fungus were studied on the host as well as in the culture grown on PDA. The associated fungus was identified up to species level based on the morphological characters, micrographs of fungus were taken using research microscope to describe the morphology of the fungi.

3.2.4 Maintenance of culture

The pure culture of *Alternaria* sp. was maintained at 4 ^{o}C in the refrigerator and it was periodically sub cultured at an interval of 15 days during the course of investigation.

3.2.5 Pathogenicity test

For pathogenicity test on the marigold plant, one month old healthy plants of susceptible cultivars were transplanted in plastic pots containing sterilized soil. Then these plants were cleaned with sterilized distilled water using moist cotton. Later plants were sprayed with a spore suspension culture by the means of fine hand atomizer. The plants without inoculum served as a check. After inoculation, the plants were watered regularly. The plants which were inoculated were encompassed with perforated polythene sheets which were moistened by sprinkling with sterilized distilled water to maintain the humidity for rapid development of symptoms. Inoculated plants were constantly observed for the appearance of disease symptoms and the incubation

period. The pathogenicity test of the causal organism was confirmed after the establishment of Koch's postulates.

3.2.6 Molecular identification of the pathogen

Isolation of genomic DNA from fungi

Total genomic DNA from the fungi was isolated by N- Cetyl- N, N, N-trimethyl- ammonium bromide (CTAB) method.

Chemicals and reagents

Extraction buffer Stock Solution	Buffer composition
1 M Tris HCl	100 mM Tris HCl
1M EDTA	100 mM EDTA
4 M NaCl	1.4 M NaCl
	1 % CTAB

- Proteinase K - 0.03 µg/µl
- SDS 20 % w/v
- Chloroform: isoamyl alcohol (24:1)
- Isopropanol
- Ethyl alcohol 70 % v/v

DNA isolation protocol

- 0.5 g fungal mycelium was taken and grinded with 25 mg PVPP using mini grinder and then centrifuged at 10000 rpm for 2 min. at 4^{o}C.
- The pellet was washed with sterile distilled water. (Centrifuge at 1000 rpm 20 min. at 4^{o}C).
- 675 µl of extraction buffer was added and incubated at 37°C for 30 min.

- 75 µl of SDS (20 %) was added and incubated at 65°C for 2 hours.
- Centrifuged at 10000 rpm for 10 min at 4°C and clear solution was collected in a sterile micro centrifuge tube.
- Equal volume of Phenol: chloroform: isoamyl alcohol (25:24:1) was added to the supernatant.
- Centrifuged at 10000 rpm for 10 min. at 4°C.
- Equal volumes of Chloroform: Isoamyl alcohol (24:1) was added.
- Centrifuged at 10000 rpm for 10 min. at 4°C
- The aqueous phase was removed and collected in a sterile micro centrifuge tube.
- 300 µl of isopropyl alcohol was added and incubated at room temperature for 1 hour.
- Centrifuged at 10000 rpm for 10 min and pellet was washed in 500 µl of 70 % ethanol.
- Centrifuged at 10000 rpm for 10 min at room temperature.
- Pellet was dried and dissolved in 20 µl sterile distilled water and stored overnight at 4^{o}C for further use.(Sambrook and Russell, 2001)

PCR Amplification: The polymerase chain reaction is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Oligonugleotide primers

Oligonucleotide	Sequences (5'- 3')	GC %	Tm value	Length bp	Product size
ITS 1	CTTGGTCATTTAGAGGAAGTAA	36.36	54.66℃	22	300 bp
ITS 2	GCTGCGTTCTTCATCGATGC	55.00	59.35℃	20	

Reagents and optimal PCR reaction mixture

PCR amplification of ITS region was done in 20 µl of reaction mixture containing PCR buffer, 1X (Kappa, SA); $MgCl_2$, 3 mM; dNTP mix, 0.25 mM; *Taq* DNA polymerase, 0.05 U; primer, 1 picomol and template DNA, 50 ng. Sterile nuclease free water was used as negative control.

PCR components	Volume (µl)
Nuclease free water	10.75
10X reaction buffer with $MgCl_2$ (1.5 mM)	2.00
dNTP mix (2.5 mM)	2.00
Primer F (10 picomoles/ µl)	2.00
Primer R (10 picomoles/ µl)	2.00
Taq DNA polymerase (5U)	0.25
Template DNA (50 ng/ µl)	1.00
Total volume	**20.0**

PCR amplification was performed in a thermal cycler with the temperature profiles of 94 ^{o}C for 2 min for initial denaturation, followed by 35 cycles of denaturation at 94 °C for 40 sec, with the constant annealing at 55 °C for 30 sec and extension at 72 ^{o}C for 1 min with the final extension at 72 °C for 5 min.

3.3 Cultural and physiological studies on isolated pathogen

3.3.1 Effect of different solid media on growth of *Alternaria alternata*

The cultural characteristics for the pathogen were examined on 12 synthetic, semi synthetic and non synthetic media. Fifteen ml of each medium was poured to each Petri plate separately. Such plates were aseptically inoculated with 5 mm disc of 10 days old actively growing culture at the center of the plates and incubated at 28±2 ^{o}C in BOD incubator for seven days. Each treatment was replicated thrice adopting a

completely randomized design. The colony diameter was recorded and the data on radial growth were interpreted statistically.

List of Media used in the study

Sl. No.	Treatments
1	Corn Meal Agar
2	Czapek's Dox Agar
3	Carrot Agar
4	Glucose Peptone Agar
5	Host Leaf Extract Agar
6	Malt Extract Agar
7	Oat Meal Agar
8	Potato Dextrose Agar
9	Rose Bengal Agar
10	Richard's Agar
11	Sabourauds's Agar
12	Waksman Agar

3.3.2 Growth phase of *Alternaria alternata*

Fifty ml of potato dextrose broth was prepared in 100 ml of the conical flask. After sterilization, these flasks were inoculated with a 5 mm disc of ten day old pure culture and incubated at room temperature of 28 ±1 ^{o}C. A set of three flasks was harvested at every 48 hr. commencing from two days after inoculation. Cultures were filtered through Whatman filter paper. The mycelial mat on the filter paper was thoroughly washed with distilled water to leach out any salts associated with the

mycelium. Subsequently, the filter papers along with the mycelial mat were dried to constant weight and weighed on an electronic balance. The data were interpreted statistically.

3.3.3 Growth of *A. alternata* on liquid media

The growth character was also observed in twelve different liquid media. All the prepared liquid media were autoclaved at 121.6 ^{o}C at 15 psi for 20 min. To carry out the study, ten days old mycelial discs of 5 mm diameter were transferred aseptically into sterilized 100 ml flasks consisting of 30 ml of the respective medium. They were incubated at 27 ± 1 ^{o}C for 10 days. Each treatment was replicated thrice. After ten days of incubation, the mycelial mat was harvested by using Whatman No. 1 filter paper and washed thoroughly with distilled water. It was dried at 40 ^{o}C for one day in a hot air oven and dry mycelial weight was recorded. The data on the dry mycelial weight was interpreted statistically.

3.3.4 Effect of pH on the growth of *A. alternata*

Effect of different pH levels on the growth of the pathogen was tried at 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 by keeping potato dextrose broth as a basal medium. The pH of the liquid medium was adjusted using 0.1 N alkali (NaOH) or 0.1 N acid (HCl). The conical flask (100 ml) consisting of 50 ml of basal medium was inoculated with the culture. Three replications were maintained for each treatment. Dry mycelial weight of the fungus was recorded and data were interpreted statistically.

3.3.5 Effect of temperature on the growth of *A. alternata*

Growth of the pathogen was examined at various temperatures, *viz.*, 15, 20, 25, 30, 35 and 40 ^{o}C. Fifty ml of potato dextrose broth was discharged into a 100 ml conical flask and sterilized. Five mm diameter of ten days old mycelial disc of the culture was incubated at different temperatures and each treatment was replicated thrice. Cultures were filtered through Whatman No. 1 filter paper and dry mycelia weight was recorded and data were interpreted statistically.

3.4 *In vitro* and *in vivo* evaluation of fungicides, botanicals and bioagents for the management of leaf spot disease

3.4.1 *In vitro* evaluation of fungicides against *A. alternata*

Efficacy of 10 selective fungicides with concentrations of 0.05, 0.1 and 0.15 per cent for systemic fungicides and 0.1, 0.2 and 0.3 per cent for non-systemic fungicides was tested by adopting poisoned food technique (Nene and Thapliyal, 1993). The required quantity of fungicides was added separately into the flasks containing molten and sterilized PDA to get the required concentration of various fungicides on the basis of their weight. Poisoned twenty ml of medium was discharged into sterilized Petri plates. Plates were inoculated in the center with 5 mm mycelial bits of the vigorously growing 7 days old culture of the *Alternaria alternata.* A control treatment was also maintained in which only sterilized water was added to the PDA. The inoculated plates were incubated at 25±1 °C in BOD incubator till fully covered mycelium growth was achieved in the control treatment.

Data on radial growth of the fungus were recorded and per cent mycelial growth inhibition was calculated as described by Vincent (1947).

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

Where,

I = Per cent Inhibition (%)

C = Growth of the test fungus in control (mm)

T = Growth of the test fungus in treatment (mm)

List of fungicides used in the study

Sl. No	Common Name	Trade Name
	Contact fungicide	
1	Captan 50 WP	Captan
2	Chlorothalonil 75 WP	Kavach
3	Copper oxychloride 50 WP	Blitox
4	Mancozeb 75 WP	Indofil M-45
	Systemic fungicide	
6	Carbendazim 50 WP	Bavistin
7	Difenoconazole 250 SC	Score
8	Hexaconazole 5 EC	Cantaf
9	Propiconazole 25 EC	Tilt
	Combination product	
9	Carbendazim 12 %+ Mancozeb 63 % WP	Saaf
10	Tebuconazole 50 % + Trifloxystobin 25 % WG	Nativo

3.4.2 *In-vitro* evaluation of botanicals against *A. alternata*

In vitro screening of six botanicals with a concentration of 5 and 10 per cent against the isolated pathogen was carried out using Poisoned food technique. For extract preparation, fresh leaves/ bulb/ kernel of healthy plants were collected and washed thoroughly with tap water and air dried. One hundred grams of plant tissue was ground using a pestle and mortar by adding an equal amount of sterilized distilled water (1: 1, w/v). The extract was squeezed through the muslin cloth and the extracts were centrifuged at 10,000 rpm for 5-10 min. The supernatant was separated and used for the experiment. An appropriate amount of extract was added to molten PDA to get desired concentrations of 5 and 10 % and shaken well for thorough mixing of the extract. The PDA plates consisting of the plant extracts were inoculated aseptically with a five mm diameter mycelial disc from fresh culture. Three replications were maintained for each

treatment. The basal medium without any plant extract served as a control. All the inoculated Petri plates were incubated at 25±1 °C. The radial growth of the test fungus was measured after attaining complete growth in the control. The per cent inhibition of fungal growth was determined using the following formula given by Vincent (1947).

List of botanicals used in the study

Sl. No.	Botanical name	Common name	Plant part used for extraction
1	*Allium sativum* L.	Garlic	Bulb
2	*Azadirachta indica Juss*	Neem	Kernel
3	*Calotropis gigantica*	Yekki/ Calotropis	Leaf
4	*Prosopis julifera L*	Bellary jali/ Prosopis	Leaf
5	*Lantana camera*	Lantana	Leaf
6	*Eucalyptus globules*	Eucalyptus	Leaf

3.4.3 *In vitro* evaluation of bio-agents against *A. alternata*

Antagonistic microorganisms like *Bacillus subtilis*, *P. fluorescens* and *T. harzianum* were evaluated for their antagonistic properties against *Alternaria alternata* by dual plate technique. Twenty ml of PDA was discharged into sterile Petri plates, after the solidification, the pathogen at one side of the Petri plate and the antagonist at the exact opposite side of the same plate was inoculated by leaving 3 - 4 mm gap. The plates were incubated for seven days at 27 ± 10 ºC. After incubation, the colony diameter of test fungus was recorded. The percentage of mycelial inhibition was determined using the formula given by Vincent (1947) as mentioned earlier.

3.4.4 *In vivo* evaluation of fungicides, botanicals and bioagents against leaf spot disease

Effective fungicides, botanicals and bioagents under *in vitro* condition were evaluated in field condition during cropping season 2018 to find out the effective treatments against Alternaria leaf spot of marigold. The experiment was laid out in a randomized block design with three replications and eight treatments. The efficacy of

each treatment was compared with a control plot, which was sprayed with water only. Two sprays of the fungicides were taken, first at the time of initiation of the disease symptom and second at fifteen days after the first spray. After each spray of the treatments, five plants were selected randomly in each treatment and disease severity was recorded by following 0-5 scale as mentioned earlier. Per cent disease index was calculated by using the formula given by Wheeler (1969).

$$\text{Per cent Disease index (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of observations x Highest disease grade}} \times 100$$

Experimental details:

Place : College of Horticulture, Bagalkot

Experimental design : RCBD

Gross plot size : 3.6 m x 2.7 m

Spacing : 60 x 45 cm

Treatments : 8

Replication : 3

Variety : Arka bangara

Effective treatments imposed against Alternaria leaf spot disease

Sl. No.	Treatments	Concentrations / dosage (%)
T_1	Copper oxychloride 50 WP	0.3 %
T_2	Mancozeb 75 WP	0.3 %
T_3	Hexaconazole 5 EC	0.1 %
T_4	Propiconazole 25 EC	0.1 %
T_5	Carbendazim 12 %+ Mancozeb 63 %	0.3 %
T_6	*Prosopis* leaf extract	5 %
T_7	*Tricoderma harzianum*	0.5 %
T_8	Control (water spray)	

4. EXPERIMENTAL RESULTS

The experiments were conducted on various aspects of Alternaria leaf spot of marigold including survey, symptomatology, morphological and molecular identification, pathogenicity test, cultural, physiological characterization and management of the leaf spot. The results of the investigation on Alternaria leaf spot of marigold are presented in this chapter.

4.1 Survey for leaf spot severity in major marigold growing areas of North Karnataka

The roving survey was conducted to assess the severity of Alternaria leaf spot of marigold during 2018 in major marigold growing areas of Northern Karnataka *viz.*, Bagalkot, Belgavi, Haveri and Koppal. In each district, two taluk were selected and in each taluk two villages were selected and in each village two fields were surveyed to assess the severity of the disease. The data pertaining to the survey is presented in Table 1.

In the Bagalkot district, the per cent disease index (PDI) ranged from 27.33 to 39.20. The highest PDI of 39.20 was recorded in kaladgi Field-2 village of Bagalkot taluk followed by Hireshellikeri Field–1 (38.40), Banashankari Field-2 (37.66) and Banashankari Field-1 (36.00) of Badami taluk. Among the four villages, Banashankari recorded the maximum PDI of 36.83 followed by Hireshellikeri (36.80). The least PDI of 27.33 was recorded in Konkanakoppa village Field-2.

In the Belgaum district, the Per cent disease index varied from 28.80 to 42.40. The highest PDI of 37.90 was observed in Hukeri taluk followed by Raybag (33.00). Among the four villages, Hanchinal recorded the maximum PDI of 40.00 followed by Awargol (35.80). The least per cent disease index was observed in Nagaral (32.80).

In Haveri district, the PDI varied from 34.33 to 57.60. The maximum PDI of 57.60 per cent was recorded in Thotadaellapura Field-1 of Haveri taluk, Devihosur Field-1 (54.40), Thumbinakatti Field-2 (48.80) of Ranebennur taluk. Among the villages, Devihosur recorded the highest PDI of 52.00 followed by Thotadaellapura

(46.40). The less PDI of 34.33 was observed in Guthla village (Field-1), Ranebennur taluk.

In Koppal district, the Per cent disease index ranged from 30.60 to 43.20. Yalburga taluk (38.82) recorded the highest per cent disease index followed by Koppal (37.45). Among the villages, Benakal recorded the maximum PDI of 40.00 followed by Bevinahalli (39.60). The least per cent disease index was observed in Munirabad (35.30).

The data summarized in the Table 1 showed that none of the surveyed locations were free from disease and the disease index varied from 30.00 to 52.00 per cent. Among the four districts surveyed, maximum mean disease severity of Alternaria leaf spot was recorded in Haveri (45.12 %) followed by Koppal (38.14 %), Belgaum (35.45 %) whereas average, minimum mean disease severity was recorded at Bagalkot (34.51 %) district.

4.2 Symptomatology

During the course of the survey and in experimental field studies under a natural condition, typical symptoms of the leaf spot of marigold were observed. The initial typical symptoms were observed as a minute brown to deep brown, circular to oblong, necrotic spots on the older leaflets towards their margin. Later, the adjacent spots coalesced with one another in order to form large irregular patches to give the blighted appearance of the leaves. Even the concentric rings were also observed in some bigger old spots. Under the severe infection, the lesions extended to stem, calyx and petals resulting in darkening, shriveling and drying of petals. The lesions on the stem were linear brown in color which later coalesced with one another to form large irregular patches. Some leaves with high severity of spots drooped and withered, which caused the death of the whole plant (Plate 1).

4.3 Pathogenicity Test

Artificial inoculation to the marigold plant with the isolated pathogen was carried out as explained in Materials and Methods.

Table 1. Severity of Alternaria leaf spot of marigold in different districts of North Karnataka

District	Taluk	Village		Area (Acre)	Spacing (cm)	Variety	Method of irrigation	Crop stage (months)	PDI	Average PDI	Cropping pattern
Bagalkot	Badami	Banashankari	Field-1	1	60×45	Local yellow	Furrow	3	36.00	36.83	Monocropping
			Field-2	4	60×30	Kolkata yellow	Furrow	2½	37.66		Monocropping
		Konkanakoppa	Field-1	0.5	60×30	Local	Furrow	1½	30.40	30.00	Monocropping
			Field-2	2	60×30	Local (orange)	Furrow	2½	27.33	**33.42**	Monocropping
	Bagalkot	Hireshellikeri	Field-1	½	60×40	Local	Furrow	2	38.40	36.80	Monocropping
			Field-2	1	60×30	-	Furrow	2½	35.20		Monocropping
		Kaladgi	Field-1	4	60×60	Indo American variety	Drip	2	29.60	34.40	Mixed
			Field-2	2	60×40	-	Furrow	3	39.20	**35.60**	Monocropping
District average										**34.51**	

Contd….

District	Taluk	Village		Area (Acre)	Spacing (cm)	Variety	Method of irrigation	Crop stage (months)	PDI	Average PDI	Cropping pattern
Haveri	Haveri	Devihosur	Field-1	3	60×60	Local yellow	Furrow	2½	54.40	52.00	Monocropping
			Field-2	1	60×40	-	Furrow	2½	49.60		Monocropping
		Thotadaellapura	Field-1	1½	60×60	Local	Furrow	2	57.60	46.40	Monocropping
			Field-2	2	60×40	Local	Furrow	3	35.20	**49.20**	Monocropping
	Ranebennur	Guthla	Field-1	½	60×60	-	Furrow	2½	34.33	36.77	Monocropping
			Field-2	2	60×60	Local	Furrow	3	39.20		Monocropping
		Thumbinakatti	Field-1	1½	60×60	Local	Furrow	3	42.40	45.60	Monocropping
			Field-2	2	60×45	-	Furrow	2	48.80	**41.19**	Monocropping
District average										**45.12**	

District	Taluk	Village		Area (Acre)	Spacing (cm)	Variety	Method of irrigation	Crop stage (months)	PDI	Average PDI	Cropping pattern
Belgaum	**Hukeri**	Awargol	Field-1	½	60×40	-	Furrow	3	39.20	35.80	Monocropping
			Field-2	1½	60×40	Local	Furrow	3½	32.40		Monocropping
		Hanchinal	Field-1	1	60×45	Kolkata yellow	Furrow	3	37.60	40.00	Monocropping
			Field-2	3	60×60	Local	Furrow	3	42.40	**37.90**	Monocropping
	Raybag	Koligudd	Field-1	4	60×60	Indo American variety	Furrow	3½	30.40	33.20	Monocropping
			Field-2	1	60×60	Local	Furrow	3	36.00		Monocropping
		Nagaral	Field-1	½	60×40	-	Furrow	2½	28.80	32.80	Monocropping
			Field-2	2	60×60	-	Furrow	3	36.80	**33.00**	Monocropping
District average										**35.45**	

District	Taluk	Village		Area (Acre)	Spacing (cm)	Variety	Method of irrigation	Crop stage (months)	PDI	Average PDI	Cropping pattern
Koppal	Koppal	Bevinahalli	Field-1	½	60×45	-	Furrow	2	42.40	39.60	Monocropping
			Field-2	2½	60×40	Local	Furrow	3	36.80		Monocropping
		Munirabad	Field-1	½	60×40	Arrow gold	Furrow	3	30.60	35.30	Monocropping
			Field-2	1	60×30	Local	Furrow	3½	42.40	**37.45**	Monocropping
	Yalburga	Benakal	Field-1	1½	60×40	Kolkata yellow	Furrow	3	36.80	40.00	Monocropping
			Field-2	½	60×60	Local	Furrow	2½	43.20		Monocropping
		Narasapura	Field-1	2	60×45	Arrow gold	Furrow	3	33.60	37.63	Monocropping
			Field-2	½	60×40	Local	Furrow		41.60	**38.82**	Monocropping
District average										**38.14**	

The pathogen produced the symptoms within 8 days after inoculation. The symptoms were first observed on the older leaves of the plants, whereas the younger leaves were the last to be infected by the pathogen. However, the development and spread of the disease occurred gradually from the bottom to the top of the plants. As the disease infection increased, the symptoms were also observed on a stem, calyx and petals. The pathogen was re-isolated from the infected leaves and the morphological characters of the re-isolated organisms were compared with the original culture of the fungus and it was confirmed that the causal agent of the disease is *Alternaria alternata* (Plate 2).

4.4 Identification of the pathogen

The morphological and cultural characters of the fungus grown on potato dextrose agar (PDA) were studied for the purpose of identification.

4.4.1 Cultural characters

The colony of *A. alternata* on PDA attained a diameter of 90 mm within 10 days. The optimum temperature for growth of the pathogen was found to be at (27 ± 2°C) with profuse mycelial growth. The initial color of mycelium was whitish in color but gradually turned light greyish and brown to dark greyish in color and produced clear cut zonations. A whitish crystal formation was also observed on the mycelial mat (Plate 3).

4.4.2 Morphological characters

The fungus produced profuse mycelial growth on PDA, which gradually turned light greyish and brown to dark greyish in color within 10 days. The mycelium was hyaline, which turned to grey brownish, multicelled and irregularly branched. The hyphae were narrow, hyaline, septate and light brown in color. Conidiophores arrised singly or in clusters and they were pale brown, in color straight or curved, in appearance geniculate, slightly swollen at apex having terminal scars showing the point of attachment of conidia. Conidia were borne in chains on conidiophores or singly. They were light to dark brown in color, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal

and 2-10 transverse septa. The muriform conidia inclusive of the beak measured 35.4 to 40.3 µm long and 7.69 to 10.04 µm wide (Plate 4).

4.4.3 Molecular identification of the isolated pathogen

In order to identify *Alternaria* sp. at the species level, molecular detection was done by using ITS oligonucleotide primer pairs ITS1 and ITS2. Agarose gel electrophoresis of PCR amplified products resulted in an amplification of 300 bp in isolated pathogen (Plate 5).

To confirm the molecular identity of the rDNA-ITS region of the present isolate was amplified and sequenced. It was then used to carry out BLAST with the nr database of NCBI gene bank database and based on 100 per cent identity score, 10 sequences were selected for preparing the phylogenetic tree using CLUSTER OMEGA programme. In the constructed phylogenetic tree analysis, it was thus confirmed that the fungal stain isolated *Alternaria alternata* was similar to *Alternaria alternata* (MK174977) (Fig 1).

4.5 Cultural and physiological studies on isolated pathogen

4.5.1 Effect of solid media on the growth of *Alternaria alternata*

The growth performance of *Alternaria alternata* was studied on twelve different solid media as described in Materials and Methods. The radial growth of the fungus was measured and the results are presented here under the Table 2 & 3 and Plate 6, Fig. 2.

The data revealed that potato dextrose agar medium had significantly the highest growth (86.67 mm) of the pathogen at eight days of the incubation followed by oat meal agar medium (82.33 mm). The minimum growth of the pathogen was recorded on the rose bengal agar (55.67 mm). Besides variation in growth rate, *A. alternata* exhibited little variation in color also were white color was observed in case of oat meal agar, Richard agar exhibited as grey at the center with white at the margin and dark brown to black in carrot agar whereas rest of the media colony color varied from grey to dark grey. The colony margin of the pathogen varied from uniform to wavy. Black pigmentation was found in Potato dextrose agar, Rose

Table 2. Growth of *A. alternata* on different solid and liquid media

Sl. No.	Treatments	Mean Radial Growth (mm)	Mean Dry Weight (mg)
1	Corn Meal Agar	82.00	47.00
2	Czapek's Dox Agar	56.67	156.00
3	Carrot Agar	76.66	304.00
4	Glucose Peptone Agar	73.00	65.00
5	Host Leaf Extract Agar	72.33	102.33
6	Malt Extract Agar	74.33	185.67
7	Oat Meal Agar	82.33	212.00
8	Potato Dextrose Agar	86.67	359.67
9	Rose Bengal Agar	55.67	152.67
10	Richard's Agar	71.33	121.33
11	Sabourauds's Agar	63.33	221.33
12	Waksman Agar	83.00	173.33
	S. Em±	**1.47**	**1.05**
	CD @ 1 %	**4.32**	**3.10**

Table 3. Morphological characteristics of *A. alternata* on different solid media

Sl. No.	Treatments	Colony character				Sporulation	Spore size (µm) (L x B)
		Colony color	Surface	Margin of the colony	Pigmentation		
1	Corn Meal Agar	Grey	Smooth	Uniform	No	+	35.12 – 39.16 x 7.80 – 9.45
2	Czapek's Dox Agar	Grey /whitish with dark border	Smooth	Irregular	Dark brown	+	34.6-38.5 x8.20 – 12.02
3	Carrot Agar	Dark brown to black	Rough	Uniform	Black	++	24.10 – 29.69 x 7.22–9.09
4	Glucose Peptone Agar	Grey with dark at the centre	smooth	Uniform	Dark brown	++	31.60 –35.38 x 7.00–09.22
5	Rose Bengal agar	Dark grey	Rough	Irregular	Black	+	29.72 – 38.00 x 6.84 – 7.89
6	Host Leaf Extract Agar	Dark grey	Smooth	Uniform	Black	+++	37.67 – 45.12 x 10.02–12.22
7	Malt Extract Agar	Light brown	Rough	Irregular	Black	++	26.15 –35.10 x 6.80–8.45
8	Oat Meal Agar	white	smooth	Irregular	Hazel	+++	36.60-37.24 x 6.47-9.8
9	Potato Dextrose Agar	Dark Grey	Smooth	Uniform	Black	++++	35.58-40.8 x 7.69-10.04
10	Richard's Agar	Whitish grey	Smooth	Uniform	Hazel	+	34.5-37.10 x 7.12-8.80
11	Sabouraud's Agar	Grey	Smooth	wavy	Hazel	+++	33.15-38.71 x 6.15-7.90
12	Waksman Agar	Grey	Smooth	Regular	Black	++	34.84-36.25 x 7.80-9.75

bengal agar, Host leaf extract agar, Malt extract agar, Waksman agar, Carrot agar, whereas Hazel pigmentation was found in oat meal agar, Richard Agar, Sabouraud's agar and dark brown pigmentation was seen in Czapek's dox agar and glucose peptone agar, but there was no pigmentation in case of corn meal agar. Potato dextrose agar supported the best sporulation. The next best media was found to be oat meal agar and Sabouraud's agar and Host leaf extract agar. The sporulation was moderate on rest of the media.

4.5.2 Effect of liquid media on the growth of the pathogen

The growth of the fungus in the different liquid media/ broth was studied as described in Material and methods. The dry weight of the mycelial growth of the fungus was measured and the results are presented here under the Table 2, Plate 7, and Fig 3.

Potato dextrose broth medium produced significantly the highest dry weight growth (359.67 mg) of the fungus, followed by Carrot broth (304.00 mg) and Potato dextrose broth was significantly superior over all other media. Corn meal broth supported the least growth (47.00 mg) of the fungus.

4.5.3 Growth phase of *A. alternata*

The experiment was conducted to determine the progress of the pathogen growth on liquid media over time. For this experiment, liquid medium that supported the good growth of *A. alternata* was selected to record the dry mycelial weight of the pathogen. Potato dextrose broth which supported the good growth was selected to assess the dry mycelia weight of the pathogen.

The dry weight of the pathogen was observed to increase after every two days after inoculation. Maximum dry weight (323.33 mg) of *A. alternata* was recorded on the 14^{th} day after incubation at 27 ± 2℃. Later the growth started decreasing at 16^{th} day of incubation (279.00 mg) and this way it went on decreasing up to 189.90 mg as observed at 20^{th} day of incubation as shown in Table 4.

4.5.4 Effect of hydrogen ion concentration (pH) on the growth of the pathogen.

In the present experiment, the effect of different levels of pH 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 were evaluated to know the optimum pH required for the growth of *A. alternata* as explained in Materials and methods.

The results in the Table 5 depict that pH range from 4.0 to 9.0 supports the growth of the pathogen. Maximum growth of the *A. alternata* was recorded at pH 6.0 (329.30 mg) which was significantly superior to all other pH levels followed by pH 7.0 (278.00 mg). The least growth was observed at pH 4.00 (150.67 mg) (Plate 8, Fig 4).

4.5.5 Effect of temperature on growth of the pathogen

In the present investigation, the effect of different temperature levels *viz.*, 15, 20, 25, 30, 35 and 40 ^{o}C were evaluated to know the optimum temperature required for the growth of *A. alternata* as explained in Material and methods.

Growth of the *A. alternata* varied significantly with different temperature levels. Results indicate that *A. alternata* grew at a wide range of temperature ranging from 15 to 40 ^{o}C. But the maximum mean dry mycelial weight (296.67 mg) was observed at 30 ^{o}C which is considered to be optimum for the better growth of the pathogen followed by 25 ^{o}C (219.00 mg) after fourteen days of incubation. The least growth of the pathogen was observed at 40 ^{o}C (42.33 mg) (Table 6, Plate 9 and Fig 5).

4.6 *In vitro* evaluation of fungicides, bio-agents and botanicals for the management of leaf spot disease

4.6.1 *In vitro* evaluation of fungicides

The efficacy of four non-systemic and two combination fungicides were evaluated at 0.1, 0.2, 0.3 per cent concentrations under the laboratory condition against leaf spot pathogen as described in Materials and methods**.**

The data (Table 7, plate 10 and Fig 6) revealed that there was significant difference between the fungicides tested. The efficacy was found to be increased in all the fungicides

Table 4. Growth phase of *A. alternata* in liquid media

Sl. No	Days after inoculation	Mean dry mycelial weight (mg)
1	2	29.27
2	4	60.03
3	6	96.67
4	8	110.67
5	10	186.00
6	12	250.33
7	14	323.33
8	16	279.00
9	18	221.00
10	20	189.90
S. Em ±		**1.60**
CD @ 1 %		**4.77**

Table 5. Effect of pH levels on mycelial growth of the *A. alternata*

Sl. No	pH levels	Mean dry mycelial weight (mg)
1	4	150.67
2	5	224.33
3	6	329.3
4	7	278.00
5	8	201.67
6	9	157.33
S. Em ±		**1.32**
CD @ 1 %		**3.56**

Table 6. Effect of temperature on mycelial growth of the *A. alternata*

Sl. No	Temperature (ºC)	Mean dry mycelial weight (mg)
1	15	99.33
2	20	191.67
3	25	219.00
4	30	296.67
5	35	127.33
6	40	42.33
S. Em ±		**1.25**
CD @ 1%		**3.89**

when concentration was increased. Copper oxychloride observed as highly effective by recording mean mycelial inhibition of 83.45 per cent which was found to be statistically superior over all other fungicides. Carbendazim 12 % + mancozeb 63 % (SAAF) and captan were the next best fungicides by recording mean mycelial inhibition of 79.99 and 76.17 per cent respectively. The least inhibition of mycelial growth was observed in chlorothalonil (59.63 %).

At 0.1 % concentration, Copper oxychloride recorded the highest mycelial inhibition of 78.51 per cent followed by captan (74.44). At 0.2 % Copper oxychloride followed by carbendazim 12 %+ mancozeb 63 % (SAAF) were highly effective by recording mean per cent inhibition of 83.33 per cent and 78.14 per cent respectively. At 0.3 % per cent, inhibition was maximum in carbendazim 12 %+ mancozeb 63 % (SAAF) followed by copper oxychloride by recording 89.25 and 88.51 per cent respectively. Whereas, least inhibition was recorded by chlorothalonil, 57.03, 60.74 and 61.10 per cent at all the concentration tested.

The per cent inhibition of radial growth of *A. alternata* by four systemic fungicides was evaluated at 0.05 %, 0.1 % and 0.15 % and presented in Table 8, Plate 11 and Fig 7.

Table 8 revealed that difenoconazole, hexaconazole, propiconazole recorded 100 per cent inhibition at all the concentration tested *i.e.,* 0.05, 0.1, 0.15 per cent and hence recorded mean inhibition of 100.00 per cent. The least mean inhibition of mycelial growth was observed in carbendazim by recording 72.66 per cent.

4.6.2 *In vitro* evaluation of botanicals against the *A. alternata*

Antifungal activity of six botanicals was assayed at two concentrations using the poison food technique as described in Materials and methods.

The results obtained Table 9 showed that all the plant extracts significantly controlled the growth of *A. alternata.* Prosopis leaf extract recorded the highest mean inhibition of 100 per cent which was superior over all other botanicals followed by Garlic bulb extract (89.45 %) and Neem seed kernel extract (89.08 %). The least inhibition was observed in Eucalyptus leaf extract (58.15 %) (Plate 12, Fig 8).

4.6.3 *In vitro* evaluation of bio control agents against the *A. alternata*

The antagonistic activity of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma harzianum* were assayed against the pathogen by dual plate technique as explained in Materials and methods.

The results in the Table 10, Plate 13 and Fig 9 revealed that *Trichoderma harzianum* was most effective in inhibition the mycelia growth of *A. alternata* with 66.89 per cent. The least mycelial inhibition was observed in case of *Pseudomonas fluorescens* with 10.67 per cent.

4.7 Field evaluation of fungicides, botanical and biocontrol agents

An experiment was carried out during Sept-Dec 2018 using five fungicides, one bioagent and one botanical for the management of Alternaria leaf spot under field conditions. All treatments were imposed twice at 15 days interval, first spray at the time of initation of the disease symptom. The data on per cent disease index and yields were subjected to statistical analysis and results are presented in table 11 and 12. The results thus obtained revealed that the treatments differ with respect to per cent disease index and yield (t/ha).

Table 11, Plate 15 and Fig 10 revealed that all the treatments significantly reduced the disease severity of leaf spot as compared to untreated control. After 1st spray, Hexaconazole @ 0.1 % recorded (27.33) least per cent disease index which was on par with propiconazole (27.73). The next best treatment was Mancozeb @ 0.3 % (31.93 PDI) followed by copper oxychloride @ 0.3 % (33.07) and *Trichoderma harzianum* @ 0.5 % (33.80) which was on par with each other. The untreated control (water sprayed) recorded maximum PDI (49.50).

With respect to per cent disease reduction over control, maximum disease reduction was recorded by hexaconazole @ 0.1 % (44.79 %) followed by propiconazole @ 0.1 % (43.98 %). The least was noticed in Prosopis leaf extract @ 5 % (22.28).

The data obtained after 2nd spray revealed that there is a significant difference between the treatments, hexaconazole @ 0.1 % recorded 23.73 per cent disease index which is significantly the least over all the treatments but on par with propiconazole @ 0.1 % (25.21

Table 7. *In vitro* efficacy of contact and combination fungicides against mycelial growth of *A. alternata*

Sl. No	Treatments	Percent inhibition of mycelia growth			
		Concentrations			
		0.1%	0.2%	0.3%	Mean
1	Captan 50 WP	74.44 (59.62)*	75.18 (60.10)	78.88 (62.62)	76.17 (60.78)
2	Chlorothalonil 75 WP	57.03 (49.03)	60.74 (51.18)	61.10 (51.40)	59.63 (50.54)
3	Copper oxychloride 50 WP	78.51 (62.37)	83.33 (65.88)	88.51 (70.16)	83.45 (66.14)
4	Mancozeb 75 WP	64.07 (53.16)	72.22 (58.17)	76.29 (60.85)	70.86 (57.39)
5	Carbendazim 12 % + Mancozeb 63 % WP	72.59 (58.40)	78.14 (62.10)	89.25 (70.84)	79.99 (63.78)
6	Tebuconazole 50 % + Trifloxystobin 25 % WG	72.59 (58.41)	74.07 (59.37)	78.14 (62.10)	74.93 (59.96)
7	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Mean	59.89 (48.71)	63.38 (50.97)	67.45 (53.99)	
Source				**S.Em ±**	**CD @ 1%**
Treatments				**0.47**	**1.34**
Concentration				**0.31**	**0.88**
Treatments x Concentration				**0.81**	**2.32**

*Figures presented in parenthesis are angular transformed values

Table 8. *In vitro* efficacy of systemic fungicides on mycelial growth of *A. alternata*

Sl. No.	Treatments	Percent inhibition of mycelial growth			
		Concentrations			Mean
		0.05%	0.1%	0.15%	
1	Carbendazim 50 WP	71.92 (57.99)*	72.75 (58.52)	73.30 (58.88)	72.66 (58.47)
2	Difenconazole 250 SC	100 (89.71)	100 (89.71)	100 (89.71)	100 (89.71)
3	Hexaconazole 5 EC	100 (89.71)	100 (89.71)	100 (89.71)	100 (89.71)
4	Propiconazole 25 EC	100 (89.71)	100 (89.71)	100 (89.71)	100 (89.71)
5	Control	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)
	Mean	74.38 (65.23)	74.55 (65.34)	74.66 (65.41)	
Source				**S. Em ±**	**CD @ 1%**
Treatments				**0.063**	**0.179**
Concentration				**0.048**	**0.139**
Treatments x Concentration				**0.108**	**0.310**

*Figures presented in parenthesis are angular transformed values

Table 9. *In vitro* efficacy of botanicals against *A. alternata*

Sl. No.	Common name	Per cent inhibition of mycelial growth		
		5%	10%	Mean
1	Calatropis leaf extract	58.15 (49.67)*	100.00 (90.00)	79.08 (62.80)
2	Eucalyptus leaf extract	51.11 (45.62)	65.19 (53.82)	58.15 (49.70)
3	Garlic bulb extract	78.89 (62.63)	100.00 (90.00)	89.45 (71.00)
4	Lantena leaf extract	65.92 (54.27)	100.00 (90.00)	82.96 (65.60)
5	Neem seed kernel extract	78.15 (62.12)	100.00 (90.00)	89.08 (70.70)
6	Prosophis leaf extract	100.00 (90.00)	100.00 (90.00)	100 (90.00)
7	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.Em ±		**0.84**	**0.37**	**0.60**
CD@ 1 %		**2.57**	**1.14**	**1.86**

*Figures presented in parenthesis are angular transformed values

Table 10. *In vitro* bioefficacy of bioagents against mycelia growth of *A. alternata*

Sl. No	Treatments	Percent inhibition of mycelia growth
1	*Bacillus subtilis*	32.20 (55.06)*
2	*Pseudomonas fluorescens*	10.67 (33.46)
3	*Trichoderma harzianum*	66.89 (19.16)
4	Control	0.00 (0.00)
S.Em ±		**0.57**
CD @ 1%		**1.722**

*Figures presented in parenthesis are angular transformed values

Table 11. Management of Alternaria leaf spot of marigold using fungicides, botanical and bioagent under field condition

Sl. No.	Treatments	Percent Disease index		Reduction over control (%)	Percent Disease index	Reduction over control (%)	Yield (t/ha)
		Before spray	After 1st spray		After 2nd spray		
1	Copper oxychloride 50 WP @ 0.3 %	38.93 (38.59)*	33.07 (35.08)	33.19	28.73 (32.39)	47.12	7.16
2	Mancozeb 75 WP @ 0.3 %	34.63 (36.03)	31.93 (34.39)	35.49	29.02 (35.58)	46.58	6.46
3	Hexaconazole 5 EC @ 0.1 %	37.23 (37.58)	27.33 (31.49)	44.79	23.73 (29.12)	56.32	8.25
4	Propiconazole 25 EC @ 0.1 %	36.80 (37.32)	27.73 (31.76)	43.98	25.21 (30.13)	53.56	7.85
5	Carbendazim 12 %+ Mancozeb 63 % WP @ 0.3 %	39.40 (38.86)	37.07 (37.48)	25.11	34.17 (35.75)	37.10	6.53
6	Prosopis leaf extract @ 5 %	39.83 (39.11)	38.47 (38.30)	22.28	33.33 (35.25)	38.65	6.11
7	*Tricoderma harzianum* @ 0.5 %	36.83 (37.35)	33.80 (35.53)	31.71	30.83 (33.71)	43.25	5.67
8	Control	36.67 (37.25)	49.50 (44.69)	-	54.33 (47.47)	-	4.42
S. Em±		**0.92**	**1.10**		**0.96**		**0.39**
CD @ 5 %		**2.83**	**3.40**		**2.93**		**1.20**

*Figures presented in parenthesis are angular transformed values

Table 12. Economics of management of Alternaria leaf spot of marigold

Sl. No.	Treatments	Cost of cultivation (Rs/ha)	Cost of fungicides (Rs/ha)	Total cost (Rs/ha)	Gross returns/ha (Rs)	Net returns/ha (Rs)	B:C ratio
1	Copper oxychloride 50 WP @ 0.3 %	46966	1890	48856	107445	58589	2.20
2	Mancozeb 75 WP @ 0.3 %	46966	1212	48178	96900	48722	2.01
3	Hexaconazole 5 EC @ 0.1 %	46966	360	47326	123795	76469	2.62
4	Propiconazole 25 EC @ 0.1 %	46966	1500	48466	117705	69239	2.43
5	Carbendazim 12 %+ Mancozeb 63% WP @ 0.3 %	46966	2196	49162	97905	48743	1.99
6	Prosophis leaf extract @ @ 5 %	46966	0	46966	91605	44639	1.95
7	*Tricoderma harzianum* @ 0.5 %	46966	500	47466	85095	37629	1.79
8	Control	46966	0	46966	66255	19289	1.41

Note: Average price of marigold flower- Rs.15/kg

per cent). Copper oxychloride @ 0.3 % recorded as the next best treatment with 28.73 per cent. The highest per cent disease index was recorded in control (54.33).

With respect to per cent disease control maximum was noticed in hexaconazole @ 0.1 % (56.32) followed by propiconazole @ 0.1 % (53.56). The least per cent disease control was recorded in carbendazium 12 % + mancozeb 63 % @ 0.3 % (37.10).

The data with respect to yield revealed that hexaconazole @ 0.1 % recorded the highest yield (8.25 t/ha) followed by propiconazole @ 0.1 % (7.85 t/ha) and copper oxychloride @ 0.3 % (7.16 t/ha), which were significantly superior over untreated control even in the size and appearance of the flowers. The untreated control recorded the lowest yield of 4.42 t/ha.

The economics of different fungicidal treatments was also studied and presented in the Table 12, hexaconazole @ 0.1 % proved to be the most economical treatment (BC ratio= 2.62) followed by propiconazole @ 0.1 % (BC ratio= 2.43) and copper oxychloride @ 0.3 % (2.20). It is evident from the present investigation that Alternaria leaf spot of marigold can be effectively managed by the two sprays of hexaconazole @ 0.1 % or propiconazole @ 0.1 % at 15 days interval under Bagalkot condition with the Net returns of Rs. 76469/- and Rs. 69239/- over the check respectively.

5. DISCUSSION

Marigold is one of the important commercial annual flower crops of India in terms of cultivation and utilization. Though having repellent property, marigold is also affected by number of fungal diseases. The important fungal disease of marigold is leaf spot and flower blight (*Alternaria* spp). It is one of the most serious and destructive diseases which often assumes epidemic proportion in farmers fields causing 50 -60 % of economic losses (Cotty *et al.,* 1983).

5.1 Survey for leaf spot severity in major marigold growing areas of North Karnataka

A detailed survey was undertaken in a major marigold growing area of North Karnataka *viz.,* Bagalkot, Belgaum, Haveri and Koppal and recored information on the per cent disease index of Alternaria leaf spot of marigold. It is evident from the survey that the incidence of disease varied from locality to locality depending on the type of variety cultivated and management practices. The incidence of the disease was also dependent on a load of inoculum and environmental conditions prevailing in different localities during cropping season. Among the four districts surveyed, maximum mean disease severity of Alternaria leaf spot was recorded in Haveri (45.12 %) followed by Koppal (38.14 %), Belgaum (35.45 %) whereas, minimum mean disease severity was recorded at Bagalkot (34.51 %) district. The survey revealed that the disease incidence found varied with respect to growing areas, varities and weather factors. The leaf spot severity and flower blight incidence recorded as 51 and 83 per cent, whereas infected seeds reduced the seed weight and germination by 28 and 54 per cent, respectively as reported by Dhiman and Arora (1990).

The Alternaria leaf spot of marigold was severe in Haveri district than in other districts. This could be because of favorable environmental conditions (High rainfall), cultural practices and initial inoculum prevailed. Also, it was attributed for the continuous growing of the crop which helped in perpetuation of the pathogen. This might have helped in the rapid development of the disease.

Least incidence of the disease was observed in Bagalkot district. This might be due to the late planting of Marigold, as under late sown condition the temperature rises

and humidity decreases during the flowering period which are not favorable for the development of the disease.

5.2 Pathogenicity test

Pathogenicity test showed the positive relation after seven days of inoculation by expressing the symptoms such as small dark brown necrotic lesion along the leaf margins on the older leaves, later advancing towards inward. Slowly lesions coalesced to form large irregular patches covering the entire leaf surface accomplished by drying up of leaves.

Wenneker *et al.* (2006) have proven the pathogenicity of *A. alternata* on the detached dormant flower bud of pear and showed pronounced death after 7 days on susceptible plants. Inoculated leaves of turmeric plants with a spore suspension of the pathogen produced symptoms in susceptible plants after 6 days of inoculation. Similar findings were reported by Ranasingh *et al.* (2006) and Thippeswamy *et al.* (2006) against *A. solani* which was found pathogenic to seedling, mature fruits and leaves of brinjal in inoculation test.

5.3 Identification of the pathogen

The fungus produced profuse mycelial growth on Potato dextrose agar (PDA), The fungus gradually turned light greyish and brown to dark greyish in color within 10 days. The mycelium was hyaline, which turned to grey, brownish, multicelled and irregularly branched. The hyphae were narrow, hyaline, septate and light brown in color. Conidiophores arise singly or in clusters and they were pale brown, in color straight or curved, in appearance geniculate, slightly swollen at apex having terminal scars showing the point of attachment of conidia. Conidia were borne in chains on conidiophores or singly. They were light to dark brown in color, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal and 2-10 transverse septa. The muriform conidia inclusive of the beak measured 35.4 to 40.3 μm long and 7.69 to 10.04 μm wide. The morphological characters observed were identical with the description reported by Karlatti and Hiremath (1989) and Keissler (1912).

5.3.1 Molecular identification

DNA sequencing has greatly enhanced the ability to accurately and reliably identify the fungi. Fungal taxonomists have been using DNA sequences for many years as a basis for the re-classification of all fungal taxa and have more recently moved to ITS sequencing as the “Gold Standard”. In this present investigation, the ITS region of halotolerant fungus was sequenced and resulted in the strain identified as *Alternaria alternata*.

In this study, the *Alternaria alternata* showed the maximum similarity with the reference ITS, and the accession number notified as MK174977. This result is in agreement with Guo *et al*. (2004), who reported the three unknown isolates of *Alternaria* species through ITS of rDNA.

5.4 Cultural and physiological studies of an isolated pathogen

5.4.1 Effect of solid and liquid media on the growth of the pathogen

Every living being requires food for its growth and reproduction. Fungi secure food and energy from the substrate upon which they live in nature. In order to culture the fungi in the laboratory, it is necessary to furnish those essential elements and compounds in the medium which are required for their growth and other life processes. Neither all media are equally good for all fungi nor there can a universal substrates or artificial medium on which all fungi grow well. So, different media including synthetic and non-synthetic were tried for the growth of *A. alternata.*

The data revealed that potato dextrose agar medium had significantly the highest growth (86.67 mm) of the pathogen at eight days of the incubation followed by oat meal agar medium (87.83 mm). The minimum growth of the pathogen was recorded on the Rose Bengal agar (55.67). Besides variation in growth rate, *A. alternata* exhibited little variation in color also were white color was observed in case of oat meal agar, Richard agar exhibited as grey at the center with white at the margin and dark brown to black in carrot agar whereas rest of the media colony color varied from grey to dark grey. The colony margin of the pathogen varied from uniform to wavy. The present finding is in conformity with Devappa and Thejakumar (2016). It is concluded that PDA has a

simple formulation and more nutrient contents, supporting the best mycelial growth of the fungus.

Potato dextrose agar supported the better sporulation. The next best media were found to be oat meal agar, Sabouraud's agar and Host leaf extract agar. The sporulation was moderate on the rest of the media. These results were in contradiction to the findings of Reddy *et al.* (2019), who found maximum radial mycelia growth of the *A. alternata* (90 mm) in PDA with excellent sporulation (++++) and poor sporulation was observed in Czapek's dox agar.

While studying, the growth of the fungus in the above mentioned same media without the agar, *i.e.,* liquid/broth media; it was found that Potato dextrose broth produced significantly highest dry weight growth (359.67 mg) of the fungus, followed by carrot broth (304.00 mg). Corn meal broth supported the least growth (47.00 mg) of the fungus. The observations are in contradictory with the results of Kantwa *et al.* (2006), who observed the maximum growth and sporulation on potato dextrose broth.

This variation in the growth of fungi in all the media is because of the composition of different media and a source of carbon used by them. For the growth of *A. alternata,* maltose, dextrose, and xylose were significant carbon sources Patil and Suryawamshi (2015).

5.4.2 Effect of hydrogen ion concentration (pH) on the growth of the pathogen

Fungi generally, utilize substrates in the form of solution only if the reaction of a solution conducive to fungal growth and metabolism. This brings the importance of hydrogen ion concentration for better fungal growth. Of all the six pH levels, maximum growth of the *A. alternata* was recorded at pH 6.0 (329.30 mg) which was significantly superior to all other pH followed by pH 7.0 (278.00 mg). The least growth was observed at pH 4.00 (150.67 mg). The pH below six and above seven was noticed to be inhibitory to the growth. The results of experiment indicated that *A. alternata* prefers a pH range of 6.00- 6.50. This showed that the fungus prefers acidic pH for its growth. The results obtained in the present study are in accordance with the results of Taware (2014), who reported that pH 6.5 was best for the growth of *Alternaria carthami* and Odenapur

(2011) observed that pH 6.0 was better for *A. alternata*. The inhibitory action of pH above 7.0 and below 6.0 was attributed to the unconducive reaction of the media.

5.4.3 Effect of temperature on the growth of the pathogen

Temperature is the most important physical environmental factor for regulating the growth and reproduction of fungi. Growth of the *A. alternata* differed significantly with different temperature levels. Results indicated that *A. alternata* grew at a wide range of temperatures ranging from 15 to 40 ^{o}C. But the maximum mean dry mycelia weight (296.67 mg) observed at 30 ^{o}C which is considered being optimum for better growth of the pathogen followed by 25 ^{o}C (219.00 mg) after fourteen days of incubation. The least growth of the pathogen was observed at 40℃ (42.33 mg). From the study, it was found that temperature ranging from 25 – 30 °C was better for the growth of *A. alternata*. The results are supported by Hubballi (2010), who reported that 30 °C was the optimum temperature for the growth of *A. alternata*.

5.5 *In vitro* evaluation of fungicides

In the absence of resistant cultivars, the use of fungicides to manage the disease is an old-age practice. When there is an outbreak of an epidemic for any reason, perhaps the use of fungicides is one of the best options available. These fungicides have to be used judiciously according to the need and kind of organism involved. Availability of new fungicides necessitates an evaluation of fungicides under *in vitro* conditions to know their efficacy and initiate spray schedule in the field conditions.

The efficacy of four non-systemic and two combination fungicides was evaluated at 0.1, 0.2, 0.3 per cent concentrations. The results revealed that copper oxychloride observed as a highly effective fungicide by recording mean inhibition of 83.45 per cent. Carbendazim 12 % + mancozeb 63 % (SAAF) and captan were the next best fungicides by recording mean per cent inhibition of 79.99 per cent and 76.17 per cent respectively. The least inhibition of mycelial growth was observed in chlorothalonil with 59.63 per cent. Prasad and Naik (2003) reported the copper oxychloride as one among the non-systemic fungicides effective in inhibiting the growth of *A. solani* and *A. alternata.* Mantecon (2007) reported that the most effective control of *A. solani* was achieved by copper oxychloride (64.7 %) followed by mancozeb (61.7 %).

Copper oxychloride has a multisite activity. It interferes with several of the (fungus) vital life functions. For this reason, resistance is less likely to develop. Copper oxychloride is a protectant fungicide/bactericide which prevents infection on plants. Its mode of action is by interfering with the enzyme system of spores and mycelium, a process that is usually irreversible. It forms a chemical barrier against fungal attack and is a foliar fungicide with preventative action.

The per cent inhibition of radial growth of *A. alternata* by four systemic fungicides was evaluated at 0.05 %, 0.1 %, 0.15 % and the results revealed that difenoconazole, hexaconazole, propiconazole recorded 100 per cent inhibition at all the concentration tested. The least mean inhibition of mycelial growth was observed in carbendazim by recording 72.66 per cent. The results are in agreement with Sangeetha (2014) who reported that hexaconazole, propiconazole and hexaconazole 4 % + zineb 68 % at all the concentrations (0.05 %, 0.075 % and 0.1 %) were proved to be the most effective fungicides in inhibiting the mycelia growth. Murthy and Shenoi (2001) also reported that Score (difenconazole), Tilt (propicanazole) and indofil M-45 (mancozeb) were potent in inhibiting the mycelial growth even at 100 ppm concentration against *A. alternata*. The result was further supported by ArunKumar (2008) who reported that propiconazole and hexaconazole were best at all concentrations (0.1 %, 0.2 % and 0.3 %) which completely inhibited the mycelial growth. Efficacy of these fungicides were also reported by Patil (2003); Wagh *et al.* (2017); Patel (2003).

Triazole antifungal agents inhibit the ergosterol biosynthesis pathway via the inhibition of 14-α-demethylase, the enzyme that removes the methyl group at position C -14 of precursor sterols. Inhibition of this enzyme leads to the accumulation of aberrant sterol intermediates (14-α- methylsterols) on the fungal surface, which results in the arrest of the fungal growth.

5.6 *In vitro* evaluation of Plant extracts against pathogen

To find out the possibilities of replacing fungicides with other eco- friendly products for the management of leaf spot disease, plant extracts of Calatropis, Eucalyptus, Garlic, Lantana, Neem, Prosopis were tested *in vitro* against the mycelia growth of *A. alternata.* The results revealed that Prosopis leaf extract recorded the highest mean inhibition of 100 per cent which is subsequently followed by Garlic bulb

extract and Neem seed kernel extract by recording mean inhibition of 89.45 and 89.08 per cent respectively. The least inhibition was observed in Eucalyptus leaf extract (58.15 %). The results were in agreement with Lingaraj *et al.* (2018), who reported that antifungal activity of Prosopis leaf aqueous extract recorded highly significant antifungal activity.

Prosopis juliflora found to contain alkaloids, flavonoids, terpenoids, tannins, sugars, and amino acids. These secondary metabolites of the plant are reported to possess good antimicrobial, anti-inflammatory, anti-malarial and growth inhibitory activities. The different groups of alkaloids isolated from this plant are reported to possess significant antibacterial and antifungal activity which is comparable to the standard drugs (Raghavendra *et al.,* 2010).

5.7 *In vitro* evaluation of bio control agents against the *A. alternata*

In the present investigation, the antagonistic activity of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma harzianum* were assayed. *T. harzianum* was found effective in inhibition of the mycelial growth of *A. alternata* with 67.23 per cent. The least mycelial inhibition was observed in case of *P. fluorescens* with 30.55 per cent. The results were in agreement with Ganie *et al*. (2013), who repoted that *T. harzianum* caused maximum mycelial growth inhibition of 71.85 % in dual culture, followed by *T. viride* (65.93 %) in *A. solani.* The results are in accordance with the findings of Waghe *et al*. (2015); Hariprasad *et al.* (2018).

The antagonism of *Trichoderma* spp. against many fungi is mainly due to production of acetaldehyde compound (Robinson and Park, 1996 and Dennis and Webster, 1971). Though, the genus *Trichoderma* comprises a large number of species some of which act as biological control agents through one or more mechanisms. Sharma *et al.* (2012) reported that *Trichoderma* strains exert control against fungal phytopathogens either indirectly by competing for nutrients and space, modifying the environment condition, promoting plant growth, plant defensive mechanisms, and antibiosis, or directly by mycoparasitism. Activation of each mechanism implies the production of specific metabolites, such as plant growth factors, hydrolytic enzymes, siderophores, antibiotics, and permeases. Specific strains of fungi in the genus *Trichoderma* colonize and penetrate plant root tissues and initiate a series of

morphological and biochemical changes in the plant, considered to be part of the plant defense response, which subsequently leads to induced systemic resistance. Antibiosis occurs during interactions with other microorganisms involving low molecular weight diffusible volatile and non-volatile toxic metabolite compounds or antibiotics like harzianic acid, alamethicines, tricholine, eptaibols, antibiotics, 6- penthylpyrone, in consideration of all these factors involving in the suppression of pathogen growth results in *Trichoderma* is an effective biocontrol agent.

5.8 Field evaluation of fungicides, botanical and biocontrol agents for management of the disease

Alternaria leaf spot is one of the serious diseases of marigold. Under favorable conditions, complete failure of the crop may occur, resulting in severe yield loss. In the absence of resistant varieties, the only effective alternative is to reduce the damage by using fungicides and biocontrol agents.

In the present investigation, all the treatments significantly reduced the per cent disease compared to the untreated control. After the first spray, hexaconazole @ 0.1 % recorded (27.33) least per cent disease index which was on par with propiconazole (27.73). The next best treatment was mancozeb @ 0.3 % (31.93 PDI) followed by copper oxychloride @ 0.3 % (33.07) and *Trichoderma harzianum* @ 0.5 % (33.80) which was on par with each other. The untreated control (water sprayed) recorded maximum PDI (49.50).

During the second spray, the results obtained revealed that there is a significant difference between the treatments, hexaconazole @ 0.1 % recorded 23.73 per cent disease index which is significantly the least over all the treatments but on par with propiconazole @ 0.1 % (25.21 per cent). Copper oxychloride @ 0.3 % recorded as the next best treatment with 28.73 per cent. The highest per cent disease index was recorded in the control (54.33). The data recorded with respect to yield revealed that hexaconazole @ 0.1 % recorded the highest yield (8.25 t/ha) followed by propiconazole @ 0.1 % (7.85 t/ha) and copper oxychloride @ 0.3 % (7.16 t/ha), which is significantly superior over untreated control. The untreated control recorded the lowest yield of 4.42 t/ha.

These results are confirmed with Mesta *et al*. (2003) and Mesta (2006) who reported that the triazoles as effective fungicides against Alternaria blight of sunflower. Shindhe *et al.* 2018 reported that hexaconazole (0.1 %) was found effective in reducing the per cent disease index (32.15) on leaves and (33.76) on a flower in all three sprays and getting higher yields (6.96 t/ha) followed by mancozeb (0.2 %) with PDI of (34.53) on leaves and (35.45) on the flower with a yield of (6.81 t/ha) as compared to control with (85.02 and 86.11 PDI) on leaves and flower respectively with the yield of (4.26 t/ha). *Trichoderma harzianum* (5 g/l) was effective in minimizing the per cent disease index (17.07) on leaves and (17.37) on flowers and recorded yield (5.26 t/ha). Singh *et al.* (2006) also obtained highest disease control and flower yield with the application of difenoconazole and penconazole.

6. SUMMARY AND CONCLUSIONS

Marigold (*Tagetes* spp.) is the one of the most important flower crop grown commercially in different parts of India. In India, it ranks first among the loose flowers. There are many constraints in the successful cultivation of this crop and among them diseases is one of the important constraints. Among different fungal diseases in marigold, Alternaria leaf spot caused by *A. alternata* is one of the most destructive diseases. Therefore, the present investigations were undertaken with the objectives to isolate and identify the pathogen causing leaf spot marigold, record its severity in different district of North Karnataka. Further, the ambit of the studies was to manage the problem of leaf spot of marigold by evaluation of different fungicides, botanicals and bioagents.

The roving survey was conducted to assess the severity of Alternaria leaf spot of marigold during 2018 in major marigold growing areas of Northern Karnataka *viz.*, Bagalkot, Belgaum, Haveri and Koppal. The results showed that none of the surveyed locations were free from disease and the disease index varied from 30.00 to 52.00 per cent. Among the four districts surveyed, maximum mean disease severity of Alternaria leaf spot was recorded in Haveri (45.12 %) followed by Koppal (38.14 %), Belgaum (35.45 %), whereas average minimum mean disease severity was recorded at Bagalkot (34.51 %) district.

The fungus associated with the Alternaria leaf spot was identified as *A. alternata* on the basis of morphological characters *i.e.* the culture of the pathogen, size and shape of the conidia of the fungus and molecular characterization by sequencing using rDNA ITS primers. The fungus produced profuse mycelial growth on Potato dextrose agar (PDA), The fungus gradually turned light greyish and brown to dark greyish in color within 10 days. The mycelium was hyaline, which turned to grey, brownish, multicelled and irregularly branched. The hyphae were narrow, hyaline, septate and light brown in color. Conidiophores arise singly or in clusters and they were pale brown, in color straight or curved, in appearance geniculate, slightly swollen at apex having terminal scars showing the point of attachment of conidia. Conidia were born in chains on conidiophores or singly. They were light to dark brown in color, varied in shape from obclavate to mostly ellipsoidal, muriform having tapered apex with 1 to 3 longitudinal

and 2-10 transverse septa. The muriform conidia inclusive of the beak measured 35.4 to 40.3 µm long and 7.69 to 10.04 µm wide.

Out of twelve different solid media tested, potato dextrose agar (PDA) proved best for the mycelial growth and sporulation (86.67 mm) followed by oat meal agar medium (87.83 mm). The minimum growth of the pathogen was recorded on the Rose Bengal agar (55.67). Among the liquid media/ broth, potato dextrose broth produced significantly highest dry weight growth (359.67 mg) of the fungus, followed by carrot broth (304.00 mg). The least growth (47.00 mg) was supported by Corn meal broth.

Out of six pH levels, maximum growth of the *A. alternata* was recorded at pH 6.0 (329.30 mg) followed by pH 7.0 (278.00 mg). The least growth was observed at pH 4.00 (150.67 mg). Growth of *A. alternata* showed the maximum mean dry mycelia weight (296.67 mg) at 30℃ followed by 25℃ (219.00 mg) after fourteen days of incubation. The least growth of the pathogen was observed at 40℃ (42.33 mg).

In vitro evaluation of four non-systemic and two combinations was taken up. Among them copper oxychloride observed as a highly effective fungicide by recording mean inhibition of 83.45 per cent. Carbendazim 12 % + mancozeb 63 % and captan were the next best fungicides by recording mean per cent inhibition of 79.99 per cent and 76.17 per cent respectively. The least inhibition of mycelial growth was observed in chlorothalonil with 59.63 per cent. Among four systemic fungicide, difenoconazole, hexaconazole, propiconazole recorded 100 per cent inhibition at all the concentration tested. The least mean inhibition of mycelial growth was observed in carbendazim by recording 72.66 per cent.

In vitro evaluation of botanicals against the mycelia growth of *A. alternata* was studied. Prosopis leaf extract recorded the highest mean inhibition of 100 per cent followed by Garlic bulb extract and Neem seed kernel extract by recording mean inhibition of 89.45 and 89.08 per cent respectively. The least inhibition was observed in Eucalyptus leaf extract (58.15 %).

The results of dual culture technique revealed that fungal bio agent were better than the bacterial bioagents in inhibiting the growth of *A. alternata*. *T. harzianum* was

found effective in inhibition of the mycelial growth of *A. alternata* with 67.23 per cent. The least mycelial inhibition was observed in case of *P. fluorescens* with 30.55 per cent.

The results of the field evaluation of fungicides, botanical and bioagent indicated that, after the first spray of hexaconazole @ 0.1 % recorded (27.33) least per cent disease index which was on par with propiconazole (27.73). The next best treatment was mancozeb @ 0.3 % (31.93 PDI) followed by copper oxychloride @ 0.3 % (33.07) and *Trichoderma harzianum* @ 0.5 % (33.80) which was on par with each other. The untreated control (water sprayed) recorded maximum PDI (49.50). The results obtained after second spray revealed that hexaconazole @ 0.1 % recorded 23.73 per cent disease index which is significantly the least over all the treatments but on par with propiconazole @ 0.1 % (25.21 per cent). Copper oxychloride @ 0.3 % recorded as the next best treatment with 28.73 per cent. The data recorded with respect to yield revealed that hexaconazole @ 0.1 % recorded the highest yield (8.25 t/ha) followed by propiconazole @ 0.1 % (7.85 t/ha) and copper oxychloride @ 0.3 % (7.16 t/ha). It is evident from the present investigation on Alternaria leaf spot of marigold can be effectively managed by two sprays of hexaconazole @ 0.1 % or propiconazole @ 0.1 % under bagalkot condition with net profit of Rs. 76469/- and Rs. 69239/- over the check respectively.

Future line of work

1. Different *Alternaria* spp. infecting marigold need to be identified with specific primers.

2. Study regarding variability of the pathogen needs to be explored.

3. Studies on toxins produced by *A. alternata* to be taken up.

4. To study the environmental factors responsible for high incidence of the disease (Epidemiological studies).

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Appendix I. Composition of the media

1. Corn meal agar

Corn meal	30 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

2. Czapek's Dox agar

Sucrose ($C_{12}H_{22}O_{11}$)	30 g
Sodium nitrate ($NaNO_3$)	2 g
Potassium dihydrogen phosphate (KH_2PO_4)	1 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.05 g
Potassium chloride (KCl)	0.05 g
Ferrous sulphate ($FeSO_4.7H_2O$)	0.01 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

3. Carrot agar

Peeled and sliced carrot	200 g
Dextrose ($C_6H_{12}O_6$)	20 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

4. Glucose peptone agar

Glucose	10 g
Bacto-peptone	2 g
di-potassium phosphate (K_2HPO_4)	1 g
Magnesium sulphate ($MgSO_4,7H_2O$)	0.5 g
Agar-agar	20 g

Distilled water (to make up)	1000 ml

5. Rose Bengal agar

Papaic digest of soybean meal	5 g
Dextrose	10 g
Monopotassium phosphate (KH_2PO_4)	1 g
Magnesium sulphate ($MgSO_4,7H_2O$)	0.5 g
Rose Bengal	0.05 g
Agar-agar	15 g
Distilled water (to make up)	1000 ml

6. Host Leaf Extract Agar

Healthy tomato leaves (green)	200 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

7. Malt Extract Agar

Malt extract	25 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

8. Oat Meal Agar

Rolled oats	40 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

9. Potato dextrose agar

Peeled and sliced potato	200 g
Dextrose	20 g

Agar	20 g
Distilled water (to make up)	1000 ml

10. Richard's Agar

Potassium nitrate (KNO_3)	10 g
Potassium monobasic phosphate (KH_2PO_4)	5 g
Magnesium sulphate ($MgSO_4,7H_2O$)	2.5 g
Ferric chloride ($FeC_{l3},6H_2O$)	0.02 g
Sucrose ($C_{12}H_{22}O_{11}$)	50 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

11. Sabourauds's Agar

Maltose	40 g
Bacto-peptone	10 g
Agar-agar	20 g
Distilled water (to make up)	1000 ml

12. Waksman Agar

Potassium monobasic phosphate (KH_2PO_4)	1 g
Magnesium sulphate ($MgSO_4,7H_2O$)	0.5 g
Glucose	10 g
Bacto-peptone	5 g
Agar	20 g
Distilled water (to make up)	1000 ml

Appendix II: Cost of cultivation of Marigold per hectare

Sl. No.	Particulars	Price (₹)
1.	Harrowing and deep ploughing	6000.00
2.	Layout and planting	4000.00
3.	Irrigation charges	3600.00
4.	weeding	4800.00
5.	pinching	1000.00
6.	Harvesting	6000.00
7.	Plantings	19,500.00
Fertilizers		
8.	NPK at 125:60:60	2066.00
	Total	**46966.00**

Appendix III. Cost of inputs used in the experiment during 2018-19

Sl. No.	Particulars	Price (₹)
Cost of Fertilizers/kg		
1.	Urea	5.52
2.	SSP	5.50
3.	MOP	17.44
Cost of chemicals/500 gm/ml		
1.	Copper oxychloride 50 WP	315.00
2.	Mancozeb 75 WP	202.00
3.	Hexaconazole 5 EC	180.00
4.	Propiconazole 25 EC	750.00
5.	Carbendazim 12 %+ Mancozeb 63% WP	366.00
6.	*Tricoderma harzianum*	50.00

Appendix IV. Rainfall statistics for NE monsoon (Oct-Dec) 2018

NAME	ACTUAL	NORMAL	DEP
States			
Karnataka	97.9	188.1	-48
MET. Subdivisions			
Coastal Karnataka			
Dakshina kannada	323.6	334.5	-3
Udupi	197.5	317.2	-38
Uttara kannada	107.1	210.4	-49
Subdivision rainfall	180.4	263.0	-31
N. I. Karnataka			
Bagalkote	28.1	145.1	-81
Belagavi	56.8	152.3	-63
Bidar	21.2	120.6	-82
Dharwad	84.4	166.2	-49
Gadag	43.4	161.6	-73
Haveri	128.9	169.9	-24
Kalaburgi	54.8	130.6	-58
Koppal	54.5	141.9	-62
Raichur	42.2	137.8	-69
Vijayapura	33.9	143.5	-76
Yadgir	22.5	153.2	-85
Subdivision rainfall	50.3	145.7	-65
S. I. KARNATAKA			
Ballari	69.6	157.0	-56
Bengaluru rural	152.3	240.7	-37
Bengaluru urban	108.6	231.6	-53
Chamarajanagar	113.0	244.8	-54
Chikaballapura	62.0	225.2	-72
Chikkamagaluru	144.8	238.5	-39
Chitradurga	121.2	159.0	-24
Davangere	115.7	176.7	-35
Hassan	131.9	221.0	-40
Kodagu	197.1	290.8	-32
Kolar	93.2	228.6	-59
Mandya	146.5	214.1	-32
Mysuru	163.5	214.3	-24
Ramanagara	136.4	243.1	-44
Shivamogga	108.2	190.4	-43
Tumakuru	124.3	205.7	-40
Subdivision rainfall	122.2	209.5	-42

ETIOLOGY AND MANAGEMENT OF BLIGHT OF MARIGOLD (*Tagetes erecta* L.) CAUSED BY *Alternaria alternata* (Fr.) Keissler

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ABSTRACT

Marigold (*Tagetes* spp.) is one of the most commonly grown and commercially exploited flower crops in India, belongs to the family Asteraceae. This crop is affected by several diseases, among them leaf spot and flower blight caused by *Alternaria* spp. is one of the most destructive disease which causes severe damage to quality and quantity of the flowers. Hence, the study on "Etiology and management of blight of marigold (*Tagetes erecta* L.) caused by *Alternaria alternata* (Fr.) Keissler was carried out at Dept. of Plant Pathology, College of Horticulture, Bagalkot during 2018-19.

During the survey, highest disease index was recorded at Haveri (45.12 %) followed by Koppal district (38.14 %), whereas least severity of disease index (34.51 %) was observe d at Bagalkot district. The pathogen associated with the leaf spot was identified as *Alternaria alternata* which was similar to the fungal strain *Alternaria alternata* (MK174977).

Among the twelve different solid media tested, Potato dextrose agar (PDA) proved best for the mycelial growth and sporulation (86.67 mm) followed by oat meal agar medium (87.83 mm). The minimum growth of the pathogen was recorded on Rose Bengal agar (55.67). Among the liquid media/ broth, potato dextrose broth produced significantly higher dry weight growth (359.67 mg) of the fungus, followed by carrot broth (304.00 mg). The least growth (47.00 mg) was supported by Corn meal broth. *A. alternata* grew well at pH 6.0 with a temperature of 30 °C.

In vitro efficacy of fungicides, botanicals and bio-agents indicated that among contact fungicides, copper oxychloride found to be a highly effective fungicide with maximum mycelial inhibition of 83.45 per cent and least inhibition was recorded in chlorothalonil (59.63). Three systemic fungicides *viz*., difenoconazole, hexaconazole and propiconazole recorded 100 per cent mycelial inhibition at all the concentration tested. Botanical, Prosopis leaf extract (5 %) and bio-agent *Trichoderma harzianum* recorded the maximum mycelial inhibition.

Field management of marigold blight with effective fungicides, botanical and bioagent indicated that two sprays of hexaconazole at 0.1 % and propiconazole at 0.1 % found effective in recording least per cent disease index of 23.73 and 25.21 with maximum net profit of Rs. 76,469/- and Rs. 69,239/- over the check respectively.

ಚೆಂಡು ಹೂವಿನಲ್ಲಿ (*ಟೆಜಟಿಸ್ ಎರೆಕ್ಟ* ಎಲ್.) *ಆಲ್ಟರ್ನೇರಿಯಾ ಆಲ್ಟರ್ನೇಟಾ* (ಫ್ರಾ.) ಕೇಸ್ಲರ್ ನಿಂದ ಉಂಟಾಗುವ ಅಂಗಮಾರಿ ರೋಗದ ರೋಗಕಾರಣಶಾಸ್ತ್ರ ಮತ್ತು ನಿರ್ವಹಣೆ

ಅರ್ಚನಾ. ಎ. ಎಮ್. **2019** **ಡಾ. ಎಂ. ಎಸ್. ಲೋಕೇಶ**
ಪ್ರಮುಖ ಸಲಹೆಗಾರರು

ಸಾರಾಂಶ

ಚೆಂಡು ಹೂವು (*ಟೆಜಟಿಸ್ ಸ್ಪಿಶೀಸ್.*) ಭಾರತದಲ್ಲಿ ಸಾಮಾನ್ಯವಾಗಿ ಬೆಳೆಯುವಂತಹ ವಾಣಿಜ್ಯ ಹೂವಿನ ಬೆಳೆಯಾಗಿದೆ. ಇದು ಅಸ್ಟೇರೇಸಿಯ ಕುಟುಂಬಕ್ಕೆ ಸೇರಿದೆ. ಈ ಬೆಳೆ ಹಲವಾರು ರೋಗಗಳಿಂದ ಬಳಲುತ್ತಿದು, ಅವುಗಳಲ್ಲಿ *ಆಲ್ಟರ್ನೇರಿಯಾ* ಶಿಲೀಂಧ್ರದಿಂದ ಉಂಟಾಗುವ ಎಲೆ ಚುಕ್ಕೆ ಮತ್ತು ಅಂಗಮಾರಿ ರೋಗ ಅತ್ಯಂತ ವಿನಾಶಕಾರಿಯಾಗಿದ್ದು, ಹೂವುಗಳ ಗುಣಮಟ್ಟ ಮತ್ತು ಇಳುವರಿಯಲ್ಲಿ ತೀವ್ರ ಕುಂಠಿತವಾಗುತ್ತಿದೆ. ಆದ್ದರಿಂದ ಪ್ರಸ್ತುತ ಸಂಶೋಧನೆಯಾದ "ಚೆಂಡು ಹೂವಿನಲ್ಲಿ (*ಟೆಜಟಿಸ್ ಎರೆಕ್ಟ* ಎಲ್.) *ಆಲ್ಟರ್ನೇರಿಯಾ ಆಲ್ಟರ್ನೇಟಾ* (ಫ್ರಾ.) ಕೇಸ್ಲರ್ ನಿಂದ ಉಂಟಾಗುವ ಅಂಗಮಾರಿ ರೋಗದ ರೋಗಕಾರಣಶಾಸ್ತ್ರ ಮತ್ತು ನಿರ್ವಹಣೆ" ಯನ್ನು ಸಸ್ಯ ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ, ತೋಟಗಾರಿಕೆ ಮಹಾವಿದ್ಯಾಲಯ, ಬಾಗಲಕೋಟೆ ಯಲ್ಲಿ 2018–19 ನೇ ಸಾಲಿನಲ್ಲಿ ಕೈಗೊಳ್ಳಲಾಗಿದೆ.

ಸಮೀಕ್ಷೆ ಕೈಗೊಂಡ ಪ್ರದೇಶಗಳಲ್ಲಿ, ಅತೀ ಹೆಚ್ಚು ರೋಗ ಸೂಚ್ಯಂಕವು ಹಾವೇರಿ ಜಿಲ್ಲೆಯಲ್ಲಿ (45.12%) ಕಂಡು ಬಂದಿದ್ದು, ನಂತರದ ತೀವ್ರತೆ ಕೊಪ್ಪಳ ಜಿಲ್ಲೆ (38.14%) ಹಾಗೂ ಕನಿಷ್ಠ ರೋಗ ತೀವ್ರತೆ ಬಾಗಲಕೋಟೆ ಜಿಲ್ಲೆಯಲ್ಲಿ (34.51%) ದಾಖಲಾಗಿದೆ. ಎಲೆ ಚುಕ್ಕೆಗೆ ಸಂಬಂಧಿಸಿದ ರೋಗಕಾರಕವನ್ನು *ಆಲ್ಟರ್ನೇರಿಯಾ ಆಲ್ಟರ್ನೇಟಾ* ಎಂದು ಗುರುತಿಸಲಾಗಿದೆ, ಈ ಶಿಲೀಂಧ್ರ ಪ್ರಬೇಧವು *ಆಲ್ಟರ್ನೇರಿಯಾ ಆಲ್ಟರ್ನೇಟಾ* (ಎಂಕೆ 174977) ಪ್ರಬೇಧವನ್ನು ಹೋಲುತ್ತದೆ. ಪರೀಕ್ಷಿಸಿದ ಹನ್ನೆರಡು ವಿಭಿನ್ನ ಘನ ಮಾಧ್ಯಮಗಳಲ್ಲಿ, ಪಿಡಿಎ ಕವಕಜಾಲ ಬೆಳವಣಿಗೆ ಮತ್ತು ಬೀಜಕಣ ಉತ್ಪಾದನೆಯಲ್ಲಿ ಉತ್ತಮವಾಗಿದೆ (86.67 ಮಿ.ಮೀ.) ನಂತರ ವೊಟ್ಮೀಲ್ ಅಗಾರ್ ಮಾಧ್ಯಮ (87.83 ಮಿ.ಮೀ) ಎರಡನೇ ಸಾಲಿನಲ್ಲಿದೆ. ರೋಸ್ ಬೆಂಗಾಲ್ ಅಗಾರ್ (55.67 ಮಿ.ಮೀ.) ಮಾಧ್ಯಮದಲ್ಲಿ ಕನಿಷ್ಠ ಕವಕಜಾಲ ಬೆಳವಣಿಗೆ ಕಂಡು ಬಂದಿದೆ. ವಿವಿಧ ದ್ರವ ಮಾಧ್ಯಮಗಳಲ್ಲಿ, ಕ್ರಮವಾಗಿ ಪಿಡಿಬಿ ಹಾಗೂ ಕ್ಯಾರೆಟ್ ಬ್ರೋತ್ ನಲ್ಲಿ ಹೆಚ್ಚಿನ ಶಿಲೀಂಧ್ರದ ಒಣ ತೂಕದ ಬೆಳವಣಿಗೆ (359.67 ಮತ್ತು 304.00 ಮಿ.ಗ್ರಾಂ) ಕಂಡುಬಂದಿದೆ. ಕಾರ್ನ್‌ಮೀಲ್ ಬ್ರೋತ್ ಕನಿಷ್ಠ ಶಿಲೀಂಧ್ರದ ಬೆಳವಣಿಗೆಯನ್ನು (47.00 ಮಿ.ಗ್ರಾಂ) ತೋರಿಸಿದೆ. *ಆಲ್ಟರ್ನೇರಿಯಾ ಆಲ್ಟರ್ನೇಟಾ* ಶಿಲೀಂಧ್ರವು 30 ಡಿಗ್ರಿ ಸೆಂಟಿಗ್ರೇಡ್ ಹಾಗೂ ರಸಸಾರ 6.0 ಇರುವ ಮಾಧ್ಯಮದಲ್ಲಿ ಚೆನ್ನಾಗಿ ಬೆಳೆಯುತ್ತದೆ.

ಪ್ರಯೋಗಾಲಯದಲ್ಲಿ ಪರೀಕ್ಷಿಸಿದ ನಾಲ್ಕು ಸ್ಪರ್ಶ ಹಾಗೂ ಎರಡು ಸಂಯೋಜಿತ ಶಿಲೀಂಧ್ರನಾಶಕಗಳ ಪೈಕಿ, ಕಾಪರ್ ಆಕ್ಸಿಕ್ಲೋರೈಡ್ ಶೇಕಡಾ 83.45 ರಷ್ಟು ಸರಾಸರಿ ಪ್ರತಿರೋಧವನ್ನು ತೋರಿಸುವುದರ ಮೂಲಕ ಹೆಚ್ಚು ಪರಿಣಾಮಕಾರಿಯಾದ ಶಿಲೀಂಧ್ರನಾಶಕವೆಂದು ಕಂಡು ಬಂದಿದೆ ಮತ್ತು ಕನಿಷ್ಠ ಪ್ರತಿಬಂಧವನ್ನು ಕ್ಲೋರೊಥಲೋನಿಲ್ (59.63 %) ತೋರಿಸಿದೆ. ನಾಲ್ಕು ಅಂತರ್ವ್ಯಾಪಿ ಶಿಲೀಂಧ್ರನಾಶಕಗಳಲ್ಲಿ, ಡೈಫೆನೊಕೊನಜೋಲ್, ಹೆಕ್ಸಾಕೊನಜೋಲ್ ಮತ್ತು ಪ್ರೊಪಿಕೊನಜೋಲ್ ಪರೀಕ್ಷಿಸಿದ ಎಲ್ಲಾ ಪ್ರಮಾಣದಲ್ಲೂ ಶೇಕಡಾ 100 ರಷ್ಟು ಪ್ರತಿರೋಧವನ್ನು ತೋರಿಸಿವೆ. ಬಳ್ಳಾರಿ ಜಾಲಿ (ಪ್ರೋಸೊಪಿಸ್) ಕಷಾಯ 5% ಹಾಗೂ *ಟ್ರೈಕೋಡರ್ಮಾ ಹಾರ್ಜಿಯಾನಮ್* ಗರಿಷ್ಠ ಕವಕಜಾಲ ಪ್ರತಿಬಂಧವನ್ನು ತೋರಿಸಿವೆ. ಕ್ಷೇತ್ರ ಪ್ರಯೋಗದಲ್ಲಿ ಶೇಕಡಾ 0.1ರ ಹೆಕ್ಸಾಕೊನಜೋಲ್ ಮತ್ತು ಶೇ. 0.1ರ ಪ್ರೊಪಿಕೊನಜೋಲ್ ಶಿಲೀಂಧ್ರನಾಶಕಗಳನ್ನು 15 ದಿನಗಳ ಅಂತರದಲ್ಲಿ ಎರಡು ಬಾರಿ ಸಿಂಪಡಿಸಿದಾಗ ಕ್ರಮವಾಗಿ ಕನಿಷ್ಠ ರೋಗ ಸೂಚ್ಯಂಕ 23.73 ಮತ್ತು 25.21 ವನ್ನು ತೋರಿಸಿರುವುದರ ಜೊತೆಗೆ ಗರಿಷ್ಠ ನಿವ್ವಳ ಲಾಭ ರೂ. 76469.00 ಮತ್ತು 69239.00 ವನ್ನು ದಾಖಲಿಸಿವೆ.