Studies on *Alternaria* blight of cabbage and its management

A Thesis submitted to the Orissa University of Agriculture and Technology in Partial fulfilment of the Requirements for the Degree of Master of Science in Agriculture (Plant Pathology)

By

SEEMA CHOUDHURY
03 PPT/12

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
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ORISSA UNIVERSITY OF AGRICULTURE &TECHNOLOGY
DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
BHUBANESWAR

Dr. S. K. Beura
Associate Professor

Bhubaneswar
Date: 23·07·2015

CERTIFICATE - I

This is to certify that the thesis entitled "**Studies on *Alternaria* blight of cabbage & its management"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Plant Pathology)** to the Orissa University of Agriculture and Technology is a faithful record of *bona fide* and original research work carried out by **Seema Choudhury** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

23/7/2015

Professor & Head
Department of Plant Pathology
College of Agriculture
O.U.A.T. Bhubaneswar

Beura 23.07.2015
CHAIRMAN
ADVISORY COMMITTEE

ORISSA UNIVERSITY OF AGRICULTURE &TECHNOLOGY
DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
BHUBANESWAR

CERTIFICATE II

This is to certify that the thesis entitled "**Studies on *Alternaria* blight of cabbage & its management**" submitted by **Seema Choudhury** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **Master of Science in Agriculture (Plant Pathology)** has been approved by the students' advisory committee and the external examiner.

Advisory Committee

Chairman

Dr. S. K. Beura
Associate Professor,

05.08.2015

Members

1. **Dr. K. C. Sahu**
Professor and Head,

5/8/2015

2. **Dr. N. K. Sahoo**
Professor,
Department of Nematology

05/8/15

5.8.15
External Examiner

Dr. R. P. NAIK
Ex- Professor & Head, P. Pathology
OUAT
(Name and Designation)

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Seema Choudhury
(Seema Choudhury)

Bhubaneswar
Date: 23/7/2015

ABSTRACT

Cabbage is one of the most important cole crops. Now it is grown almost throughout the year for its economic and nutritional value. India occupies 2nd position in cabbage production worldwide. It suffers from a wide array of fungal, bacterial and viral diseases which is causing severe loss in yield. *Alternaria brassicae*, the incitant of leaf blight of cabbage is a fungal pathogen has been reported from all the continents of the world as well as in the state of Odisha in wet seasons and in areas with relatively high rainfall. Symptomatology study revealed the seedlings developed spots on leaves leading to damping off. Initially small yellow specks are produced on leaves and stem which gradually darken and enlarge into circular, dark to tan coloured concentric rings giving a target board effect. In pure culture, the fungus produced profuse cottony mycelium, whitish to grey in colour at first and turned dark with age. The conidia are brownish black, obclavate with 4-11 transverse and 0-4 longitudinal septa measuring 28.85-67.28×11.39-13.91µm with the average of 41.90×12.94µm. Conidiophores found to be distinctly geniculate arise in fascicles, dark brown unbranched, straight with 1-5 septate. Cultural studies revealed that Potato dextrose agar or Richard's broth supported maximum growth (85.89mm & 653.41mg) of the test fungus at 10 days of inoculation .The preferred carbon & nitrogen sources for growth of *Alternaria brassicae* were found to be sucrose & L- asparagines with the mean dry weight of 492.37 & 676.67mg respectively at 10 days of inoculation. The optimum pH for the growth of the test fungus was recorded to be 7.0 corresponding to maximum dry weight growth (360mg). The plate cultures of the fungus put under 16 hours of light along with 8 hours of darkness could facilitate highest radial growth (69.97mm) followed by the plates subjected to 12 hours light along with 12 hours dark (68.92mm), both being statistically at par with each other. From management study it was revealed that among the 11 phytoextracts evaluated, leaf extract of Eucalyptus at 20% concentration was found to be superior in inhibiting the mycelial growth (76.81%). Among the natural oils, superiority of clove oil (0.5, 0.75 & 1%) established the investigation in respect of inhibition of mycelial growth of the test fungus (100%). Five biocontrol agents were tried *in vitro* against *Alternaria brassicae* in terms of the per cent inhibition of mycelial growth. *Trichoderma viride* (79.26%) found promising followed by *Trichoderma harzianum* (76.91%), both being statistically at par with each other. Nine agrochemicals were evaluated against *Alternaria brassicae* in terms of the per cent inhibition of the mycelial growth. Propiconazole at 0.15% was the best chemical resulting in significantly the maximum growth inhibition of the test fungus (100%) followed by Difenconazole showing 84.16% growth inhibition.

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INTRODUCTION

Cabbage (*Brassica oleracea L. var capitata*) is one of the most important cole crops belonging to family Cruciferae and is grown for the thickened main bud called "Head". The word cabbage is an anglicised form of the french "Cobbache" meaning head and it is mostly used as culinary and dietic articles, salad, pickles, boiled vegetables, cooked in curries, dehydrated vegetables and can also be used for feeding livestock.

The Food and Agriculture Organization of the United Nations (FAO) reports that world production of cabbage for 2014 was almost 71 million metric tons. Almost half of these crops were grown in China, where Chinese cabbage is the most popular *Brassica* vegetable. China has the 1st position in cabbage production worldwide and India is on the 2nd position. The Food and Agriculture Organization of the United Nations (FAO) reporting that total world production of all Brassicas for 2014 was 70,104,972 metric tons. The nations with the largest production were China which produced 47% of the world total and India which produced 12%. China and India used a surface area of 980,000 hectares (2,400,000 acres) and 375,000 hectares (930,000 acres) respectively.

Cabbage (*Brassica oleracea*) is a leafy green or purple biennial plant grown as an annual vegetable crop for its dense leaved heads. It descends from *B. oleracea var. oleracea*, a wild field cabbage. Cabbage heads generally range from 0.5 to 4 kilograms (1 to 9 lb) and can be green, purple and white. It is a multi-layered vegetable. Plants are 40–60 cm tall in their first year at the mature vegetative stage and 1.5–2.0 m tall when flowering in the second year. Cabbage heads average between 1 and 8 pounds (0.5 and 4 kg) with fast growing earlier maturing varieties producing smaller heads. Many shapes, colors and leaf textures are found in various cultivated varieties of cabbage. Leaf types are generally divided between crinkled leaf, loose head savoys and smooth leaf firm head cabbages while the color spectrum includes white and a range of greens and purples. Different varieties prefer different soil types ranging from lighter sand to heavier clay but all prefer fertile ground with a pH between 6.0 and 6.8.

From nutritional point of view, Cabbage is a good source of vitamin K, vitamin C and dietary fiber. Cabbage provides 25kcal in form of carbohydrate 5.8g, dietary fibre 2.5g, fat, 0.1g, protein 1.28g, vitamin B 0.671mg, vitamin C 36.6mg, vitamin K 76µg. Calcium 40mg, Iron 0.47mg, Magnesium 12mg, Manganese 0.16 mg, Phosphorus 26 mg.

Potassium170 mg, Sodium 18 mg, Zinc 0.18 mg and florate 1µg (Choudhary, 1967). Cabbage has some phytochemicals which have anti disease effects. Such compounds include sulforaphane and other glucosinolates which may stimulate the production of detoxifying enzymes during metabolism. Studies suggest that cruciferous vegetables including cabbage may have protective effects against colon cancer. Purple cabbage contains anthocyanins which are under preliminary research for potential anti-carcinogenic properties. Cabbage used juice as an antidote for mushroom poisoning. The health benefits of cabbage include frequent use as a treatment for constipation, stomach ulcers, headache, obesity, skin disorder, eczema, jaundice, scurvy, rheumatism, arthritis, gout, eye disorders, heart diseases, aging and Alzheimer's disease. Contaminated cabbage has been linked to cases of food-borne illness in humans. They can be pickled, fermented for dishes such as sauerkraut, steamed, stewed, sauteed, braised or eaten raw.

For optimal growth there must be adequate levels of nitrogen in the soil especially during the early head formation stage and sufficient phosphorus and potassium during the early stages of expansion of the outer leaves. Temperatures between 4 and 24 °C (39 and 75 °F) prompt the best growth. The cabbage crop is affected by various fungal as well as bacterial diseases like damping off, club root, downy mildew, *Sclerotinia* rot, black leg, black rot, soft rot and *Alternaria* blight or *Alternaria* leaf spot. The disease *Alternaria* leaf spot of cabbage is prevalent in all the cabbage growing states and is one of the major biotic problems, which limits its production and also quality of produce. There are two species of *Alternaria* which cause serious damage in cabbage i.e. *Alternaria brassicae* and *Alternaria brassicicola*, they can survive saprophytically out side of the host and diseased crop debris. The primary sites of survival from year to year are the resting spores (Chlamydospores, Microsclerotia).

The genus *Alternaria* was first recognised by Nees in 1817. In 1836, Berkele identified the causal fungus on plants belonging to family *Brassicaceae* as *Macrosporium brassicae* (Berk.), which was later renamed as *Alternaria brassicae*

(Berk.) by Saccardo (1886). The most common symptoms of *A. brassicae* are yellow dark brown to black circular spots with target like concentric rings. *A. brassicae* and *A. brassicicola* can affect host species at all stages of growth including seeds. On seedlings symptoms include dark stem lesions immediately after germination that can result in damping-off or stunted seedlings. The pathogens can shrivel seeds within the pods or kill the pod stalks before seed formation. They may also be a means by which bacterial soft rot enters the stem which may lead to plant death. (Chupp and Sherf,1960). In addition to destruction of a seed crop the pathogens can live within the seed spread the disease to other fields and cause a loss of seedlings (Rangel, 1945).

There are reports that the disease appeared in the first fortnight of July and maximum disease intensity was noticed when the temperature ranged in between 25 to 28°C and average relative humidity was more than 80%. Rainfall was held greatly responsible for the severity of infection and disease development (Ahamad and Narain 2000). The disease incidence could cause yield reduction up to 35-60% (Kolte *et al.*, 1987).

The *Alternaria* leaf blight disease was also observed in severe form in the economic botanist section II of the Central Research Farm, Orissa University of Agriculture and Technology, Bhubaneswar. Although the leaf blight disease is considered to be a major disease of the crop, no systemic work appears to be done on the disease in India or elsewhere.

Keeping this in view, the present investigation on *Alternaria brassicae*, the incitant of leaf blight of cabbage was undertaken in order to make a detailed study of the morphological characters and physiological behaviours of the pathogen and to find out suitable management practices for the disease under laboratory condition with following objectives:

1. Collection of disease samples, isolation of pathogen & pure culturing of pathogen.
2. Proving pathogenicity.
3. Cultural & physiological studies of the test fungus.
4. Epidemiological studies in relation to disease incidence & development.
5. Management of the disease by botanicals, bio agents & chemicals.

CHAPTER-II

REVIEW OF LITERATURE

REVIEW OF LITERATURES

Alternaria blight of cabbage caused by *Alternaria brassicae* is an important and widely distributed disease through out the world and it occurs in different parts of India. As much research work been carried out, much information is not available regarding this disease in the literature. Review was made on disease and pathogen of cabbage and the other hosts of this pathogen.

2.1 History of disease

Alternaria black leaf spot was reported in the early 20th century. Initially researcher described only few characters of disease. Later on different other researcher called it *Alternaria* blight.

Berkeley (1836) noticed fungal infection on plant belonging to the family Brassicaceae and identified this fungus as *Macrosporium brassicae* (Berk.) which was later renamed as *A. brassicae* (Berk.) Sacc by Saccardo (1886).

2.2 Causal organism

Wadhwani and Dudeja (1982) reported that the disease caused by *Alternaria brassicae* and *Alternaria brassicicola* to develop in three phases. It was reported to appear first on leaves in contact with the soil when prevalent RH was high. It spread after pollination during period of heavy rains. Leaf orientation was reported to be important in providing a platform for infection from falling contaminated petals. The last phase appeared on fruits.

Tripathi and Kaushik (1984) reported that the intensity of seed infection to vary with number of lesions per siliqua. The associations of *Alternaria brassicae* was demonstrated with seed from 3 crops of rapeseed (*Brassica campestris*) and mustard (*B. juncea*). Populations of the pathogen decreased with increasing temperature and storage temperature. *Alternaria brassicae* survived in plant debris buried in field soil at depths below 7.5 cm. *Chenopodium album* was recognised as a colateral host in the field.

Reis and Boiteux (2010) reported that the *Alternaria brassicae* and *Alternaria brassicicola* are the major leaf pathogens on Brassicaceae throughout the world. Both fungi were able to induce similar symptoms in common host.

2.3 Distribution and economic importance

Babadoost and Gabrielson (1979) found out that *Alternaria* leaf spot was caused a minor disease of seed crop of brussels and cabbage in USA.

Daebler *et al.* (1986) and Humpherson-Jones (1992) reported that disease epidemiologically spread in Germany and loss caused up to 50% on rapeseed about 28.4% loss caused in Romania due to this disease on *Brassicae spp.* (Tasca and Trandaf, 1984). It was also causing loss in broccoli and stored vegetable of Brassicae.

Kolte *et al.* (1987) reported the results of field experiments over 5 consecutive seasons in which infection by *Alternaria brassicae* and *Alternaria brassicicola* reduced 1000 seed weight and seed yield causing losses upto 46.57% in rape and 35.38% in mustard.

Tripathi *et al.* (1987) reported that *Alternaria brassicae* to cause severe yield reduction and quantitative differences in oil contents of rape and mustard crops.

Cerkauskas (1988) stated that *Alternaria brassicae* was a major pathogen of cruciferous vegetables in Ontario, Canada.

Kadian and Saharan (1993) reported heavy yield losses due to pod infection in yellow sarson (*Brassica campestris*) followed by brown sarson. They further reported that deep lesions on the pods increased seed infection and decreased pod length, seeds per pod, 1000 seed weight, seed germination and oil content. Isolates from rai, brown sarson and toria infected 7 test crops but not cabbage. Isolates from cabbage, cauliflower and radish infected all test hosts. The incubation period was shorter in rapeseed and mustard group crops compared to the vegetables. Symptoms on 15 hosts differed in size of spots (>1-20mm) and colour (yellow-black), forming of concentric rings in lesions and yellow haloes round the spots.

Verma and Saharan (1994) stated that *Alternaria* leaf spot is also causing huge amount of economic lose in different parts of Europe.

Mustard and canola crop were cultivated on large area in India but *Alternaria* leaf spot is main threat, which cause huge amount of economic loses every year (Chahal, 1981, Saharan, 1991,Singh and Bhowmik, 1985).

Verma *et al.* (1994) and Kolte (2002) reported that yield losses of up to 71.5% were recorded in *Brassica rapa* and *Brassica juncea* .

Tamayo *et al.* (1999) reported that *Alternaria brassicae* was causing head rot of cauliflower in Colombia resulting in 30% losses in South America.

Shah and Abdullah (2000) reported that *Alternaria brassicae* was one of the major pathogen of mustard and canola in some other parts of sub continent such as Pakistan and Bangladesh. Yield losses against different verities of mustard were found to range from 8.62-17.71 and 13.58-38.50 respectively in Pakistan.

Stonehouse (2000) reported that in Canada, it was more prominent disease and it varies from place to place and also from season to season but mainly it empress from rainfall during rapeseed and canola, severely damaging up to 75% of the crop. In 1990, mustard and canola cultivated on a huge area in Alberta (Canada) but *Alternaria brassicae* caused huge momentary loose more than $23 million Canadian.

Saharan *et al.* (2003) stated four species of *Alternaria* i.e. *A. brassicae, A. brassicicola, A. raphani and A. alternata*, have been reported worldwide to cause *Alternaria* blight on rapeseed and mustard but *A. brassicae* is the most predominant and economically important. All the commercial cultivars are susceptible to *Alternaria.*

Reis and Boiteux (2010) reported that *A. brassicae* and *A. brassicicola* are the major leaf pathogen on Brassicaceae throughout the world. Both the fungi frequently isolated, being prevalent on the *Brassica oleracea* complex. Where as *A. brassicae* was prevalent on the *B.rapa* complex and weed species.

2.4 Prevalence of disease

Twenty five fields were randomly surved by Tewari and Conn (1990) for prevalence of *Alternaria* black spot. They described that *Alternaira* black spot was more sever in this year as compare to last years. Further, probably this disease was more economically important disease of rapeseed across the prairies. In the heavily infested fields the yield losses from this disease was 30% more as compare to normal dockage.

Harrison and Loland (1991) was surveyed 47 fields in the peace river region. During survey he observed different diseases such as *Alternaria* black spot, root rot, and blackleg of rapeseed-canola. The prevalence and incidence of *Alternaria* black spot was range from 2 to 24% and overall 87% of field survey. Root rot was ranging from 0.68 to 3.18% and overall 1.49%. Blackleg disease prevalence and incidence was very low as compared to previous year disease trend. Therefore, the prevalence of *Alternaria* black spot was high as compare to other disease.

Rop and Kiprop (2009) conducted survey on 89 farms of *Brassica spp.* crops. After survey they found out that about 46 farms have prevalence (51.7%) of *Alternaria* black spot disease of *Brassicae spp.* The prevalence and severity of *Alternaria* black spot was high, which cross the economic threshold level of yield.

Sami (2012) conducted survey on Brassicaceae family comprises plant species that are *Brassica oleracea* and *Brassica rapa.* The occurrence and prevalence of *Alternariam sp.* causing leaf spots in Brassica crops was accessed, as well as the existence of a possible preference by vegetable host for these pathogens.

2.5 Disease symptoms

Kadian and Saharan (1984) confirmed pathogenicity on *Brassica spp.* and reported that the symptoms produced by *A. brassicae* was very prominent, formation of concentric rings in the lesion with yellow halo zone.

Kong *et al.* (1995) reported that the susceptibility of sunflower tissues against *A. helianthi* increased with age, so that the older leaves were more susceptible than young and expanding leaves. They also reported that planting density in field conditions influenced the disease development.

Hudec and Ronacik (2002) reported that symptoms caused by *Altrernaria alternata* included 0.3 to 1.0 cm oval, light brown leaf spots which darkened with age having typical concentric circles in the centre of the spots.

Saharan and Mehta (2002) reported that symptoms are first visible with appearance of black points. Later, these spots enlarge and develop into prominent round spots with concentric rings showing target board characteristic of the spot. Many spots coalesce to form large patches and causing blighting and defoliation of the leaves. In

some *Brassicas spp.* formation of constricting rings in the lesion and zone of yellow halo around the lesions are very prominent

Schwartz *et al.* (2004) described about the symptoms of *Alternaria* black spot of canola. Brown to black spots was produced on leaves which were enlarging under favorable conditions. Lesions became gray and develop a purple or brown border. Under dry condition lesions remains small and black. Under favorable condition defoliation occurs. Under continuous favorable, *Alternaria* black spot may cause seed rot and post emergence damping off.

Chaerani *et al.* (2006) found that *Alternaria solani* caused diseases on foliage (early blight) and basal stem of seedling (collar rot) of tomato.

Khan (2010) described the performance of different inoculum methods with *Alternaria brassicea* and *Alternaria brassicola* in mustard. Two cultivars Pusa bold and Rohini were used against *Alternaria brassicae* and *Alternaria brassicola*. He used four methods of inoculation such as foliar spray, agrose gel, soil and seed inoculations. After inoculation, different concentric lesions of yellow to brown color were developed on leaves. Under favorable condition severity of disease increased on leaves and then transfered to seed and stem. Foliar method was more affecting way of disease prevalence. Over all plant length was decreased due to inoculation with pathogen. Seed and soil inoculation method produced mild blight symptom.

Meena *et al.* (2010) reported that the *A. brassicae*, *A. brassicicola*, and *A. raphani* caused more or less similar symptoms on leaves, stem and siliquae of oilseed brassicas. Spots produced by *A. brassicae* appear to be usually grey in color when compared with black sooty velvety spots produced by *A. brassicicola*. Spots induced in response to *A. raphni* showed distinct yellow hallow around them. However, the symptoms may vary with the host and environment.

2.6 Isolation of the fungus

Petrie (1974) reported that *Alternaria carthami* occurred on 95% of untreated safflower seeds.

Chahal (1980) revealed that seed infection by *Alternaria brassicae* was confined to discoloured grey seeds and could not be eliminated by surface sterlization

with 2% sodium hypochloride for 10 minutes. Conidia of *Alternaria brassicae* were present at all times over fields of sarson during the growing season.

Verma and Saharan (1993) isolated and identified *Alternaria brassicae* and *Alternaria brassicicola* from rape and Indian mustard. *Alternaria raphani* was most common on radish but also occurred on other Brassicae vegetable and oil yielding crops.

Virendra *et al.* (2006) collected eleven isolates of *A. solani* from different agro climatic zones.

Zhang et *al.* (2011) isolates and identify *Alternaria brassicae* from Chinese cabbage in Bijie City & showed that the disease was caused by *Alternaria sp.* the size of its spores was (15.0-38.5)μm × (7.5-13.5)μm. Combining with the characteristics of host and pathogen the pathogen caused black disease of Chinese cabbage was identified to be *Alternaria brassicicola.*

2.7 Pathogenicity

The primary infection results from the wind borne spores produced on plant debris of previous crop or on weeds and other collateral hosts growing in the vicinity. Conidia germinate in presence of moisture giving rise to germ tube, which emerged from any cell of the spore. Germ tubes from germinated spores of *Alternaria* penetrate undamaged tissue of the Brassicaceous host directly.

Bains and Tewari (1987) reported that homodestruxin B was a compound produced by *Alternaria brassicae*. This compound is phytotoxic in nature and cause black leaf spot of rapeseed. Further, he described that another phytotoxic destruxin B was produced by pathogen when applied to mustard plant. On the other hand, homodestruxin B caused symptoms of different severities on leaves of non host plants. So, from this research he found out that homodestruxin B is a non host specific toxin which can affect wide host range.

Awasthi and Kolte (1989) *Alternaria* blight of mustard developed best during rosette to flowering stage and produced more number of spots on mustard leaves.

Khodke *et al.* (2000) studied the infected leaves of chilli and observed circular brown or black spot of various sizes with concentric zonation. *Alternaria alternata* was isolated from infected leaves confirmed the pathogenicity and concluded that *Alternaria alternata* was the causal agent of leaf spot disease of chilli.

Scholze (2004) evaluated conidial germination and pathogenicity of *A. brassicicola* isolates on four cabbage cultivars of (*Brassica oleracea* var. *capitata*: Amager, Turkis, Krautman and Toskama) and two cultivars of savoy (*B. oleracea* var. *sabauda*, Marner Grunkopf and Plainpalais).And concluded that significant differences in aggressivity as measured by the size of lesions on leaf discs.

Akhtar *et al.* (2004) observed *Alternaria alternata* incitant of leaf blight of tomato and also proved its pathogenicity.

Mangala *et al.* (2006) investigations were made on the pathogencity of *Alternaria alternata* on chilli cultivars. The fungus isolated from diseased chilli leaves produced typical leaf blight symptoms upon inoculation to healthy chilli plants that were similar to those recorded on naturally infected plants. Upon artificial inoculation small necrotic spots appeared on plant species such as tomato, red gram, black gram, green gram, groundnut, cabbage and mustard. While blighted symptoms were observed on brinjal, tobacco, soyabean, clusterbean, potato and cauliflower.

Tziros *et al.* (2008) proved the pathogenicity of *Alternaria alternata* on pomegranate fruits incitant of fruit rot of pomegranate and identifed on the basis of their morphological characteristics.

Parihar (2010) reported that metabolic activities changed in *B. juncea* from infection of *Alternaria brassicae* causing black spot disease. He selected seven genotypes (EC-399313, EC-399299, EC-399296, EC-399312, PHR-2, Varuna and EC- 399302) of Indian mustard. According to him the percentage disease severity was maximum in Varuna and EC-399302 as compare to other genotypes. With increase in infection the phenolic contents tend to increase whereas flavanols contents decreased. From *Alternaria brassicae* infection, EC-399296 and EC-399313 genotypes showed maximum increase in total soluble protein contents.

Sharma and Meena (2012) conducted a study in which he described aggressiveness and diversity of *Alternaria brassicae* isolates. He used thirty isolates of *Alternaria brassicae*. Different synthetic media showed clear variation in morphology in isolates of *Alternaria brassicae*, its required specific nutrition for aggressive growth. Due to fungicides effect, different isolates of pathogen gave maximum growth but some isolates gave very poor mycelia growth, which showed isolates aggressiveness against different fungicides.

2.8 Morphological characteristic of pathogen

Malaguti *et al.* (1972) described the pathogen isolated from necrotic tissues of leaves and stems. Conidiophores generally were thin and almost straight. Conidia were seen singly or in chains with transverse and longitudinal septa and with or without an apical rostrum. The conidia without the rostrum had a length of 41.6 to 80.0 µm and a width of 9.6 to 18.2µm (average 60.8 x 15.1 µm). The length of the rostrum varied ranging from 22.4 to 233.3 µm. In culture medium, the conidia were more irregular and smaller than those observed in nature (26 to 41 x 9 to 19 µm). Isolates obtained from leaf spots and stem striations were found to be same in morphological characters. They further reported that the leaf spot caused by *A. sesamicola* were well defined with the size of 2 to 12 mm at times, reaching up to 20 mm with concentric areas in irregular circles.

Kolte (1985) described the mycelium of *A. brassicae* is septate, brown to brownish grey in colour. The conidiophores are dark, septate arise in fascicles measuring 14-74 × 4-8 µm. Conidia are brownish black, obclavate borne singly or sparingly in chains of 2-4, muriform with long beak and the overall conidial size ranges between 148-184 × 17-24 µm with 10-11 transverse and 0-6 longitudinal septa. This species represent slow and rudimentary growth in media and forms chlamydospores in less frequency. Mycelium of necrotrophic fungus *A. brassicicola* is septate, olive grey to greyish black in colour. The conidiophores are olivaceous, septate, branched, measuring 35-45 µm in length and 5-8 µm in width. Conidia are dark, cylindrical to oblong, muriform without beak measuring 44-55 µm in length and 11-16 µm in width with 5-8 transverse and 0-4 transverse septa. The fungus grows faster in media with high sporulation and appears as well developed black sooty colony with distinct zonations.

Kiprop *et al.* (2009) described the morphological characteristics of *Alternaria* black spot disease of *Brassicae spp.* in Kenya. There were two species of *Alternaria* (*Alternaria brassicicola* and *Alternaria japonica*) responsible for it. *Alternaria brassicicola* conidia were long multi branched chain. Conidia were acropleurogenous, straight, and ovoid to nearly cylindrical and beakless. Conidia contain 7 longitudinal and up to 1-11 transverse septa. Conidia and septa have chestnut brown in edge view while reddish brown to yellowish brown from front face. On the other hand *A. japonica* conidia were short in length about 2-4 with short chain. They were beakless strongly constricted at the transverse septa, smooth-walled and mid-brown. When conidia were mature then their colour slightly dark. There were clear variation in septa such as some having1-2 longitudinal septa and 2-3 transverse septa.

Ramjegathesh and Ebenazar (2012) reported that all the isolates of *A. alternata* causing leaf blight disease of onion produced light brown conidia with muriform shape, but the isolates varied in the size (length, width and beak length) of the conidia, the number of cells per conidium and sporulation times.

Sharma *et al.* (2013) reported that the colony colour of the *A. brassicae* isolates varied from light olive grey to olivaceous black among all 32 isolates of the fungus infecting cauliflower and mustard leaves. The mycelia colour varied from brown and golden. The conidia characteristics were also similar to each other among the isolates viz. conidia colour was golden to brown with mostly smooth surface.

Giri *et al.* (2014) studied the 10 Indian isolatesof *A.brassicae* and grouped the isolates based on cultural characteristics in three groups. Group 1 isolates produces circular white colonies with a fluffy appearance with smooth colony margins. Group 2 isolates have off white colony with a feathery appearance and is circular with all type of margins. Group 3 isolates having light brown colony with cottony appearance and colony are circular in shape with wavy and rough margins.

2.9 Cultural studies

Growth of the pathogen on different culture media

Nutritional requirement of various fungi differ and there is no one medium which can be universally suited to all the fungi. Therefore, studies on the nutritional

requirement of a pathogen is an important aspect as this helps in better understanding of host pathogen relationship and disease management (Lilly and Bernett, 1951).

Rane and Patel (1956) observed the best growth of *A. macrospora* infecting cotton in Richard's liquid medium although it grew fairly well on other media tested.

Mallikarjunaiah and Rao (1972) reported the good mycelial growth and sporulation of *A. sesami* infecting sesame in Potato dextrose agar, Oat meal agar, Richard's agar and Asthana and Hawker's agar. But moderate growth was observed in french bean, corn meal, Sabouraud's and Brown's agar media.

Mathur and Sarbhoy (1977) reported on two isolates of *A. alternata* which attained maximum growth in Richard's broth followed by Czapek's Dox broth medium.

Mahabaleswarappa (1981) observed the maximum growth of *A. carthami* causing symptoms of safflower on Potato dextrose broth medium followed by Czapek's, Richard's and Sabouraud's broth media.

Osman *et al.* (1992) observed that Czapeck's Dox agar medium supported maximum growth of *Alternaria alternata* followed by Potato dextrose agar and Waksman media.

Saeed *et al.* (1995) reported that *Alternaria alternata* grew best on Richard's agar medium and maximum colony growth observed at 27°C temperature and pH 5.5.

Shekarappa (1999) reported the maximum growth of *A. sesami* infecting sesame on Richard's broth and Potato dextrose broth followed by host extract broth. They observed that the maximum radial growth of *A. sesami* causing leaf spot of sesame was observed in host extract agar; however there was no significant difference in supporting fungal growth among host extract agar, Potato dextrose agar and Carrot extract agar media. Least growth was observed in Czapek's agar medium. They observed significantly higher mycelial growth of *A. sesami*, causing leaf spot of sesame on Potato dextrose broth which increased with increase in incubation period and reached its peak at 12th day after inoculation and thereafter started declining.

Khokde *et al.* (2000) reported the most prounced growth and sporualtion of *Alternaria alternata* the incitant of chilli leaf spot on Richard's medium followed by Potato dextrose agar, Czapeck's medium and Oat meal agar medium.

Maheshwari *et al.* (2001) tested eleven nutrient media for the growth and sporulation of *Alternaria alternata* and reported maximum growth of the fungus on Potato dextrose agar followed by Oat meal agar medium.

Singh *et al.* (2001) observed that potato dextrose agar medium supported better mycelial growth and sporulation of *Alternaria alternata* followed by Richard's medium, Czapecks dox agar and Asthana medium.

Kumar and Singh (2003) studied on effects of eight nutrient media on the growth and sporulation of *A. brassicae*. The maximum growth of the fungus was observed in both solid and liquid forms of Radish root extract, followed by Carrot root extract, Potato dextrose and Oat meal agar. Poor growth was observed on Asthana and Hawker's, Richard's, Standard nutrient and Czapek (Dox) media. No sporulation was observed in any of the solid and liquid media.

Akhtar *et al.* (2004) reported that *Alternaria alternata* causing leaf blight of tomato grew well on Potato dextrose agar and formed grayish black colony of 90 mm in diameter in 7 days.

Pandey *et al.* (2006) studied the effect of various culture media on the growth, sporulation and morphological variations of *Alternaria alternata* (fr.) Keissler. Out of 10 media tested, Potato dextrose agar supported the best mycelial growth and sporulation of the test fungus followed by the Oat meal agar, Richard agar, Maize meal agar, Host leaf extract agar, Czapeck agar, Malt extract agar, Kirchoff's medium, Coon's agar and Asthana and Howker's media.

Isra Ram *et al.* (2007) observed maximum growth and sproulation of *Alternaria alternata* causing *Alternaria* fruit rot of ber on Potato dextrose agar medium.

Madhavi *et al.* (2012) had undertaken studies on *alternaria porri* and reported that Czapex-dox medium amended with nutrients like lactose, urea, diammonium hydrogen orthophosphate and ammonium sulphate supported good growth of pathogen.

Ramjegathesh and Ebenezar (2012) reported that among the solid media tested *Alternaria alternata* causing leaf blight of onion grow best in host leaf extract agar and modified Czapek's Dox medium which increased the growth of mycelium followed by Potato dextrose agar medium and Carrot agar medium.

Sharma *et al.* (2013) reported that potato dextrose agar and host leaf extract agar supported maximum mean radial growth of different isolates of *A. brassicae*, obtained from the infected cauliflower and mustard leaves among all the culture media tested. Radial growth of 14 isolates were higher on PDA medium (>80 mm) on the 7th day after inoculation.

Somappa *et al.* (2013) studied that *Alternaria solani* growth was best on Potato dextrose broth (34.1mg) followed by Czapeck's medium (58mm) and sporulation was maximum on Potato dextrose agar (13.2×10^6 spores/ml).

2.10 Nutritional studies of the pathogen

Carbon and Nitrogen Sources

Pawar and Patel (1957) observed maltose, xylose and arabinose as good sources of carbon for the growth of *A. ricini* infecting castor.

Chaturvedi (1966) reported that *A. alternata* utilized fructose, lactose, maltose and arabinose effectively.

Gupta *et al.* (1970) reported that among the eight monosaccharides tested, fructose supported maximum growth and sporulation of *A. brassicae* infecting cabbage. Further, they found that mannitol supported good growth while the growth was poor on maltose.

Goyal (1977) found that growth of *A. alternata* was maximum on maltose followed by sucrose, starch, glucose and maltose but poor growth was noticed on galactose and mannitol.

Nehemiah and Deshpande (1977) determined influence of amino acids and vitamins on *in vitro* and *in vivo* cellulose production by *Alternaria brassicae (Berk). Sacc.* Although all the aminoacids except glycine increased growth of the fungus *in vitro*, cellulose production was inhibited especially by glycine and L-lysine

monohydrochloride *in vivo,* cellulose production was reduced. All the vitamins tested simulated growth but inhibited *in vitro* cellulose production especially nicotonic and folic acids. The latter acids proved to be most inhibitory to rot development in potato and *in vivo* cellulose production.

Mathur and Sarboy (1977) reported that pentose sugars poorly supported the growth of *A. alternata* infecting sugar beet while sucrose supported maximum growth.

Mohapatra *et al.* (1977) studied on physiology of the sesame leaf blight pathogen, *Alternaria sesami* and recorded maximum growth of *A. sesami* on mannitol followed by lactose and starch.

Ansari *et al.* (1989) reported the nutritional requirements of *Alternaria brassicae* for growth and sporulation. *A. brassicae* could utilize all 'C' sources tested for growth. Maximum growth occurred on starch whereas as minimum on glycerol. Sporulation occurred in all 'C' sources.

Maganhotto and Melo (1999) reported that among various C sources tested, highest mycelial growth of *A. solani* was achieved with glucose and maltose (C) with an average ratio of 0.75 cm / day. Sporulation reached highest values with galactose and arabinose (5.0 x 103 and 4.1 x 103 conidia ml-1 respectively).

Singh (2000) reported sucrose as the best carbon source for the growth of *A. porri* infecting onion.

Hossain and Mian (2003) reported peptone and L- asparagine showed excellent performance as nitrogen sources to support the growth and sporulation of *Alternaria brassicicola*, isolated from *Alternaria* blight infected cabbage leaf with moderate mycelial density.

Madhavi *et al.* (2012) tested five carbon sources on growth of *A. porri*, pathogenic to onion. Among the carbon sources tested, lactose supported the best growth of *A. porri* followed by galactose and dextrose.

Ramjegathesh and Ebenezar (2012) reported that maltose followed by glucose, sucrose and fructose as carbon sources supported maximum growth of the 10 isolates

of *A. alternata* causing leaf blight disease of onion, collected from 10 different conventional onion growing areas of Tamil Nadu.

Devi *et al.* (2014) studied on two isolates of *A. helianthii* infecting sunflower collected from different areas of Tamilnadu and reported that glucose was found to be the best source of carbon supporting maximum growth of the most isolates of the fungus among the 6 carbon sources tested.

2.11 Physiological studies

Generally, all the fungal species prefer a temperature range of 20-30° C with neutral pH (7.0).

2.11.1 Effect of different levels of pH on the growth of test fungus

Hasija (1970) reported that optimum pH and temperature for growth and sporulation of *Alternaria alternata* was 6.6 and 25^{0}C.

Ansari *et al.* (1989) reported the effect of some factors on growth and sporulation of *Alternaria bassicae* causing *Alternaria* blight of rapeseed and mustard. Growth and sporulation of *A. brassicae* were affected by temperature, RH, pH and exposure to light and darkness. Growth and sporulation occurred at 5-30° C and were optimum at 23°C. The pathogen grew at all RH levels tested but a gradual increase in RH enhanced mycelial growth and sporulation reaching an optimum at RH 95-100%.Mycelial growth reached a maximum at pH 6.5 but occurred at all pH values tested. An increase or decrease from pH 6.5 gradually suppressed growth. Sporulation occurred at all pH levels except 2.9 and 9.2. Alternate light and dark was better for growth than continuous or complete darkness.

Ozcelik and Ozcelik (1990) studied effects of temperature and pH on biomass production by *Alternaria spp.* and reported that biomass production was maximum at 25^{0}C and pH 8.0.

Wang and Dong (1991) reported that the favourable temperature and pH for mycelial growth of *Alternaria alternata* was found to be 20-25^{0}C and pH 5.5 to 5.7 *in vitro*.

Zhu *et al.* (1996) observed the optimum pH and temperature for mycelial growth of *Alternaria tenuis* (*Alternaria alternata*) was 6.5 and 20-30^{0}C.

Joshi *et al.* (2009) showed that maximum pectolytic enzyme (which breaks pectic substances of plant tissue) production by *A. solani* the tomato early blight fungus, was recorded at pH 7.8. However, minimum enzyme production was recorded at pH 4.0.

Madhavi *et al.* (2012) studied the influence of pH on the growth of *A. porri*. Maximum fungal growth was observed in broth adjusted to pH 5.0 followed by the medium with a pH of 4.0 and 6.0. The growth was poor at pH 9.0. The fungus did not grow at highly acidic levels of pH 2.0 and 3.0. Growth of *A. porri* was found maximum at pH 5.0.

Mishra and Mishra (2012) studied on *A. alternata* in cotton and reported that maximum fungal growth (493.0 mg) was observed at pH 6.5 followed by pH of 7.0 (461.50 mg) and 6.0 (435.20 mg). The least growth weighing 72.80 and 54.0 mg were recorded at pH 2.50 and 12.0 respectively which suggested that high alkaline and acidic behaviour did not favour the growth of the pathogen.

Ramjegathesh and Ebenezar (2012) reported that low pH (4.0 to 4.5) was found ideal for the growth of *A. alternata*, while the minimum growth was observed in pH 9.0 irrespective of 10 isolates of *A. alternata* causing leaf blight disease of onion. They also reported that the maximum germination of the conidia of *A. alternata* causing leaf blight disease of onion was at 46°C irrespective of 10 isolates of the fungus.

Devi *et al.* (2014) studied on 2 isolates of *A. helianthii* causing leaf spot of sunflower collected from different areas of Tamilnadu and reported that most isolates of the fungus preferred pH of 6.96 to 7.15 when the fungus was incubated for 24 h. They also further reported that most isolates of the fungus preferred temperature of 29.22 to 33.93°C.

2.11.2 Effect of Light on growth and sporulation of test fungus

Lukens (1963) reported that the conidia of *A.solani* were normally formed after incubation for 6 hours in the dark.

Prasad and Dutt (1971) studied *A. solani* in potato and found maximum sporulation in 6 day old culture with 24 hours of exposure to sunlight than culture exposed to incandescent electric light or infrared light.

Gupta *et al.* (1972) studied the effect of light on growth and sporulation of *Alternaria brassicae* (Berk.) Sacc. Maximum growth and sporulation occurred with alternate day light and darkness, exposure to red light being next best. Sporulation was completely inhibited by continuous light.

Khandelwal (1974) observed that several members of the genus *Alternaria* have been reported to be light sensitive. In recent years much work has been done on the inhibitory and stimulating effects of visible and ultraviolet light on sprorogenesis.

Singh *et al.* (2001) studied the effect of light / darkness on germination and sporulation of *Alternaria tenuissima* and observed maximum conidial germination in total darkness followed by 8 hrs light / 16 hrs darkness and 16 hrs light / 8 hrs darkness respectively.

Prasad and Naik (2002) observed maximum growth of five isolates of *A. solani* in alternate 12 hours light and 12 hours darkness.

Changkun *et al.* (2004) observed that the growth rate of *Alternaria brassicae* was significantly higher under 24 hrs light or 12 hrs alternate light than that under 24 hrs darkness. For *Alternaria japonica* incubation under 24 hrs light or 24 hrs darkness was more efficient than that under 12 hrs alternate light.

2.12 Management studies

Vast range of research was conducted with respect to food poisoning technique against *Alternaria brassicae* under *in-vitro* conditions.

2.12.1 Studies on botanicals influencing the growth of the fungus

Shivpuri *et al.* (1997) recorded that, the fungi toxic properties of ethanol extracts of 10 plant species viz; *Allium cepa, Allium sativum, Azadirachta indica, Calotropis procera, Datura stramonium, Ocimum sanctum, Polyalthia longifolia, Tagetes erecta* and *Vinca rosea* against five pathogenic fungi i.e. *Alternaria*

brassicola, *Colletotrichum capsici*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotium*.

Singh and Majumdar (2001) tested the efficacy of plant extracts viz., *Allium sativum*, *Allium cepa*, *Curcuma longa*, *Zingiber officinale*, *Azadirachta indica*, *Datura stramonium* and *Ocimum sanctum* against *Alternaria alternata* and concluded that extract of *Allium sativum* showed minimum disease severity followed by *Ocimum sanctum* and *Zingiber officinale*.

Choudhary *et al.* (2003) studied the efficacy of leaf extracts of *Eucalyptus globolus*, *Datura stramonium*, *Solanum xanthocarpum*, *Azadirachta indica*, *Lantana camera*, *Ricinus communis* and *Lawsonia inermis*; bulb extracts of *Allium sativum*, *Allium cepa* and rhizome extracts of *Zingiber officinale* in controlling *Alternaria alternata in vitro*. The bulb extracts of *Allium sativum* recorded the highest inhibition of the pathogen (56.19%) followed by the bulb extracts of *Allium cepa* (54.27%).

Prasad and Naik (2003) reported that extracts of garlic and neem were found most effective against *Alternaria solani* causing leaf blight of tomato.

Chand and Singh (2004) were evaluated extracts of aak (*Calotropis procera*), eucalyptus (*Eucalyptus globulens*), jatropha (*Jatropha multifida*), neem (*Azadirachta indica*) and bulbs of garlic (*Allium sativum*) on *Alternaria* blight (*Alternaria brassicae*) of Indian mustard cv. RH-30 under laboratory conditions. All the extracts effectively reduced the disease. Foliar spray with bulb extract of *Allium sativum* showed the lowest disease intensity (2.87%) followed by *E. globulens* (5.3%) and *Azadirachta indica* (7.4%) compared to 20% in the control. *J. multifida* and *C. procera* were comparatively less effective than the other plant extracts but these also reduced the disease intensity from 20% to 7.5 and 11.9% respectively.

Patni *et al.* (2005) were evaluated methanol extract of six medicinal plants against *Alternaria* blight of mustard. *Eucalyptus*, ashok and *Calotropis* extracts in that order were promising in limiting the growth and sporulation of the pathogen whereas *Parthenium* extracts promoted these. The disease was adequately managed by the Eucalyptus, Ashok and *Calotropis*. Yield of mustard seeds was significantly highest in Eucalyptus spray over all the other treatments at 12.02 q/ha. The checks had only 7.35 q/ha yield. Ashok and *Calotropis* sprays also provided better control.

Gorawar and Hedge (2006) tested the antifungal activities of extracts of *Allium sativum, Azadirachta indica, Cassia occidentalis, Clerodendron inerme, Duranta repens, Ferula asafoetida, Lantana camera, Ocimum sanctum, Parthenium hysterophorus, Tridax procumbens* and *Vinca rosea* at 5 or 10% concentration against *Alternaria alternata* causing leaf blight of turmeric and observed that *Ferula asafoetida* at 10% was the most effective in the inhibition of the pathogen followed by *Azadirachta indica* seed kernel extract 10%.

Harlapur *et al.* (2006) was used thirteen plants extract, eight biological agents and twenty three fungicides against antifungal activity by food poisoning technique. Potato dextrose agar media was used for pathogen growth at different concentration. Neem extract at 10% concentration showed maximum growth inhibition (56.64%) *Aloe vera* oil extract at 10% concentration gives (53.50%) growth inhibition.

Vadilal and Ebenezer (2006) reported maximum inhibition of mycelial growth and sporulation of *Alternaria solani* with extracts of *Acorus calamus* and moderate inhibition with *Prosopis juliflora* and garlic clove.

Eight plants extract were studied by Raveesha *et al.* (2007) against antifungal activity of *Alternaria* at 10% concentration in form of food poisoning technique. The result has shown that *Decalepis hamiltonii* provided significant reduction of fungus growth. Further, dilution of *D. hamiltonii* was also used for comparison of eight species of *Fusarium*, ten species of *Aspergillus*, three species of *Penicillium* and two species of *Drechslera*. After application of these biological agents, he observed significant antifungal activity against *Alternaria*. In this study he found that *P. chrysogenum* was totally inhibiting the growth of fungus at 10% concentration. *D. halodes* and *A. fumigatus* inhibited the total growth of fungus at 20% concentration, while *F. lateritium* and *F. moniliforme* inhibited fungus growth at highest 50% concentration. Meanwhile different solvent such as petroleum, benzene, chloroform, ethanol and methanol were used as antifungal activity against *Alternaria*. Petroleum ether gave high level of antifungal activity as compare to other organic solvents whereas no antifungal activity was observed from methanol and ethanol extract at 2000 µg/ml.

Singh *et al.* (2007) tested antifungal activity of *Parthenium sp., Eucalyptus sp., Datura stramonium, Calotropis procera, Azadirachta indica, Lantana camara, Withania somnifera, Vinca rosea, Aloe vera, Ocimum sanctum, and Zingiber officinale against Alternaria brassicae* , the causal organism of *Alternaria* blight of *Crucifers*. Maximum growth inhibition of the fungus recorded with *Azadirachta indica* (67.7%) followed by *Lawsonia inermis* (63.0%), *Datura metel* (39.2%), *Calotropis procera, Lantana camara* (36.6%) and *Citrus sp* (28.1%) against leaf and bud blight disease in linseed caused by *A. lini* when aqueous leaf extracts (2.0%) of the above mentioned plants were taken.

Panchal and Patil (2009) tested the efficacy of extracts of garlic, turmeric and neem at 10% concentration against *Alternaria alternata in vitro* and reported that garlic clove extract proved highly effective in reducing *Alternaria* fruit rot of tomato followed by turmeric and neem extract.

Aslam *et al.* (2010) studied food poisoning technique against *Alternaria*. She used five medicinal plants e.g. *Capparis decidua, Dodonaea viscose, Adhatoda zeylanica, Azadirachta indica* and *Salvadora oleoides*. After the completion of work she concluded that, *D. viscose* has shown maximum effect against *Alternaria* as compared to other plants extracts followed by *A. zeylanica* (77.44%). At last *Salvadora oleoides* gave a minimum inhibition against *Alternaria*. She described in his research that with the increase in concentration of plant extract, the fungal growth reduced. The highest inhibition in colonies growth of pathogen was observed at 100 and 200g/l.

Meena *et al.* (2010) conducted an experiment *in vivo* by taking plant extracts viz; *Azadirachta indica* (neem leaf or seed kernel 10%), Nimbicidine (azadirachtin 0.03%) and leaf extracts (10%) of *Withania somnifera*, Aloe *barbadensis, Calotropis gigantia, Ocimum sanctum, Nerium odorum and Vinca rosea.* Nimbicidine spray treatment provided a good level of disease control (59%). Treatments with aqueous leaf extract *Calotropis* and *Azadirachta* as well as kernel extract were also effective in controlling blight.

Singh and Kerkhi (2010) reported maximum growth inhibition of *A. lini* infecting linseed by neem leaf extract (21.8 %) and linseed leaf extract (14.2 %).

Bhardwaj (2011) evaluated twenty plant extracts against *Alternaria brassicae* a causal organism of leaf spot diseases in *Crucifereae* and brown rot of cauliflower. The maximum inhibitory effect was shown by *Curcuma domestica* followed by *Clerodendron inerme* > *Acacia catechu* > *Anthocephalus cadamba.*

Nath *et al.* (2012) reported that leaf extracts of *Boswellia ovalifoliolata*, *Euphorbia tirucalli* and *Cassia tora*at 500ppm concentrations reduced the growth of fungus (*Alternaria alternata*) as compared to 250ppm.

Sasode *et al.* (2012) studied with different botanicals viz medicinal plants such as neem, *Eucalyptus*, Tulsi, *Lantana*, *Datura* and Pudina at 10% concentration against *Alternaria brassicae* under laboratory condition by food poisoning technique. Neem and eucalyptus used inform of oil. All the extract was effective against growth of *Alternaria brassicola* and *Alternaria brassicae* but neem crude extract at 10% concentration showed minimum growth of fungus. Therefore, neem extract showed prior effect as compared to *Eucalyptus*, Tulsi and *Lantana*. Under boil forms the minimum radial growth was also recorded in Neem. The oil extract (Neem and *Eucalyptus*) were found less effective as compared to crude and boil extracts. Three different types of treatment were used in this investigation against *Alternaria.*

Sharma and Meena (2012) evaluated highest per cent inhibition of mycelial growth of *A. brassicae* was observed the growth the growth inhibition in *Lawsonia inermis* (30.0%) followed by *Erythrina chiaposana* (25.5%), *Ricinus communis* (16.8%) and *Zingiber officinalis* (16.3%) at 5% concentration of aqueous leaf extract for controlling *Alternaria* blight disease of rapeseed and mustard.

Ganie *et al.* (2013) evaluated extracts of five plants viz. *Azadirachta indica*, *Lantana camera*, *Ocimum sanctum*, *Eucalyptus globulus* and *Calotropis gigantea* by poison food technique (PFT) @ 3, 5, 7 and 9% concentrations against *Alternaria brassicae* causing blight of mustard. *In vitro* study on *A. brassicae* revealed that all five plant extracts at all four concentrations significantly inhibited the mycelial growth of this pathogen as compared to control. However *O. sanctum* was found most efficacious with growth inhibition (31.85%) followed by *E. globulus* (28.97%) and *L. camara* (23.60%).

Jagana *et al.* (2013) reported that the disease intensity of *Alternaria* blight of mustard caused by *A. brassicae* was reduced by *Allium sativum* (ST) @ + *Allium. sativum* @ 1% (FS), *Azadirachta indica* (ST) @ 1% (w/v) + *A. indica* (FS) @ 1% (w/v). They also reported that the disease intensity of *Alternaria* blight of mustard caused by *A. brassicae* was reduced by *Trichoderma harzianum* (ST) @ 1% + *T. harzianum* (FS) @ 1 % followed by *Pseudomonas fluorescens* (FS) @ 1 %.

Singh *et al.* (2013) stated that two foliar sprays of aqueous extracts (10%) of *Azadirachta indica* seed kernel and *Calotropis procera* or *Azadirachta indica* leaves suppressed *Alternaria* blight of mustard caused by *A. brassicae* and enhanced mustard seed yield under artificial inoculation conditions.

Bhargav *et al.* (2014) reported that neem seed kernel extract was better as compared to *Ocimum cannum* and *Tridax procumbense* among the three plant extracts against *A. helianthi* laboratory condition. They also reported that *Pseudomonas fluorescens* was the most effective biological agent against *A. helianthi* infecting sunflower followed by *T. harzianum*.

Harde and Suryawanshi (2014) reported *in vitro* evaluation of 13 botanicals / plant species @10 and 20 % against *Alternaria brassicae*, inciting *Alternaria* blight of mustard (*Brassica juncea* L.). All the botanicals tested were found fungitoxic and significantly inhibited mycelial growth of *A. brassicae* over untreated control. *Azadirachta indica* (Neem) was found maximum mean growth inhibition (80. 46%) of the test pathogen. The second and third best botanicals in respect of fungitoxicity were *Polyalthia longifolia* (Ashoka) and *Ocimum sanctum* (Tulsi) which recorded next best maximum mean growth inhibition of 77.76 and 71.41% respectively. This was followed by the botanicals *viz. Lantana camera* (65. 65%) *Datura metal* (54. 34%), *Eucalyptus globulus* (53.38%) *Zingiber officinale* (48.79%), *Bougainveillia spectabilis* (46.03%), *Vinca rosea* (45.98%), *Lawsonia innermis* (43.54%), *Tridax procumbens* (34.46%) and *Allium sativum* (32.55%). Comparatively, *P. hysterophorus* (Parthenium) was found least fungitoxic and recorded minimum mean growth inhibition (16.05 %).

Waghe *et al.* (2014) reported that at 10 and 20 per cent concentration maximum inhibition was recorded with neem (63.05 and 68.88 %) this was followed

by karanj (56.38 and 63.60 %) mehandi (52.49 and 60.55 %). Minimum inhibition was recorded with *Datura* (40.55 and 48.60 %) followed by nirgudi (49.99 and 54.16 %) against *A. helianthi.*

2.12.2 Studies on oils influencing the growth of the fungus

Babu *et al.* (2000) mentioned that, spraying with 3% Neem oil in tomato pot cultures resulted in 53% reduction in disease caused by *Alternaria solani.* He also reported that the effect of plant extracts, oils and neem plant products, *Acacia concinna* pod extract resulted in the lowest per cent disease index in the field (23%) followed by Neem oil (30.9%).

Mohan *et al.* (2001) conducted an experiment on management of leaf blight diseases of onion by taking plant extracts and plant oils. They reported that lowest disease percentage was obtained with palmarosa oil at 0.1% when sprayed at first appearance of disease symptom.

Vadivel and Ebenzar (2006) reported that Palmarosa oil (0.1%), neemoil (3%) and *Madhuca indica* oil (3%) also inhibited the mycelial growth.

Hadizadeh *et al.* (2009) analyzed antifungal potential of five essential oils against *Alternaria alternata.*

Eleven essential oils (clove, rosemary, cinnamon leaf, sage, scots pine, neroli, peppermint, aniseed, caraway, lavender and common thyme) were tested for *in vitro* antifungal activity on twelve plant pathogenic fungi (*Fusarium graminearum, F. verticillioides, F. subglutinans, F. oxysporum, F. avenaceum, Diaporthe helianthi, Diaporthe phaseolorum var. caulivora, Phomopsis longicolla, P. viticola, Helminthosporium sativum, Colletotrichum coccodes and Thanatephorus cucumeris*) The best antifungal activity had common thyme, cinnamon leaf, clove and aniseed oils (Jasenka *et al*, 2010)

Yadav *et al.* (2014) reported that the neem oil with 3% concentration supported minimum disease severity on leaves (15.13%) against the growth of *Alternaria* spp, incitant of *Alternaria* leaf spot of Cabbage (*Brassica olaracea* var. *capitata* L.).

2.12.3 Studies on bio control agents influencing the growth of the fungus

Basim and Katircioglo (1990) studied the antagonistic activity of 12 isolates of *B. subtilis* against *A.* alternata and *A. solani* by dual culture technique. Among the isolates tested, *B. subtilis* AB 2 and AB 27 isolates had the most antagonistic effect against the pathogens tested. High level of bacterial antagonism by *P. fluorescens* (61 %) and *Bacillus spp* (30%) was recorded against *A. brassicicola.*

Leifert *et al.* (1992) reported that high level of bacterial antagonism by *P. fluorescens* (61 %) and *Bacillus spp.* (30%) was recorded against *A. brassicola.* Among them, *P. fluorescens* C 42, 66 and 82 isolates provided the best control of fungi.

Abdul *et al.* (2001) reported that *Trichoderma harzianum* and Nimokil 60 EC (neem oil product) was effective in vitro in inhibiting the mycelial growth of *Alternaria solani.*

Atia and Esh (2005) studied the role of biotic agent in controlling *Alternaria* fruit of tomato and pepper caused by *Alternaria alternata* and observed that bio-control agents such as *Bacillus subtilis, Pseudomonas fluorescens, Trichoderma harzianum* and *Trichoderma viride* were effective in inhibiting the mycelial growth of *Alternaria alternata.*

Harlapur *et al.* (2006) reported that different species of *Trichoderma used against Alternaria brassicae* but *Trichoderma harzianum* shown maximum antifungal activity about (65.17%) and secondly, *Trichoderma viride* showed maximum fungus growth. Against antifungal activity of *Alternaria spp*, The tested ten plant extracts with 10% concentration and four bio agents were used at 0.1% concentration. *Prosopis juliflora* showed maximum colony inhibition about 81.2% followed by *Pseudomonas fluorescens* and *Trichoderma viride* gave maximum fungal growth inhibition. Plant extracts (*P. juliflora*) and antagonists (*P. fluorescens and T. viride*) were tested against *Alternaria* under *in vivo* conditions along with fungicide Mancozeb. He defined that with the decrease the concentration of plant extract, bio agents and fungicide, the fungal growth increased and vice versa.

Kumar *et al.* (2006) evaluated efficacy of three antagonists, viz., *Trichoderma virens*, *Trichoderma harzianum* and *Trichoderma viride* against *Alternaria alternata* causing *Alternaria* leaf spot of *Vicia faba* and observed that *Trichoderma viride* was most effective against *Alternaria alternata.*

Vadilal and Ebenezer (2006) reported maximum inhibition of mycelial growth and sporulation of *Alternaria solani* with biocontrol agents like *Bacillus subtilis*, *Trichoderma viride* and *Gliocladium virens*.

Singh *et al.* (2010) reported that, *T. harzianum* (36.0 %) and *T. viride* (25.0 %) were signifi cantly reducing disease intensity of *Alternaria* leaf spot and bud blight disease of linseed crop incited by *A. lini.*

Yadav *et al.* (2014) reported that *Trichoderma viride* (5 gm./kg seed) and *Pseudomonas fluorescens* (5 gm/kg seed) found effective against the growth of *Alternaria* spp, incitant of *Alternaria* leaf spot of Cabbage (*Brassica olaracea* var. *capitata* L.)

2.12 4 Studies on chemicals influencing the growth of the fungus

Singh and Bhowmik (1885) conducted experiment on the effect of eight fungicides differed in persistence on the leaf surface when compared for inhibition of spore germination of *A. brassicae* at various time intervals. Fungicides which were more toxic were also more persistent than those with low toxicity. Generally, the rate of decline in toxicity was more rapid in the beginning than subsequently. Among the fungicides tested, Difolatan (captafol) was the most persistent and effective both in reducing leaf blight intensity and in increasing seed yield of *Brassica juncea* cv. Pusa bold. Dithane M-45 (mancozeb) was the next in performance and Syllit [dodine] and Cuman L also gave satisfactory results. Fytolan (copper oxychloride) was found phytotoxic.

Shivpuri *et al.* (1988) studied on the efficacy of some fungicides against *Alternaria* blight of mustard (*Alternaria brassicae*)and its effect on grain yield and reported that minimum disease was observed in Rovral treated plots and it was significantly superior to all the treatments followed by Captafol and Dithane M 45. Maximum yield was observed in Rovral treated plots followed by Captafol.

Dubey *et al.* (2000) tested nine fungicides against *Alternaria alternata* causing blight of broad bean and reported that Blitox-50 inhibited maximum mycelial growth followed by Kavach and Bavistin.

Kamble *et al.* (2000) tested six fungicides against tomato and brinjal leaf spot caused by *Alternaria alternata* and observed that mancozeb was found highly effective in inhibiting the mycelia growth followed by copper oxychloride and iprodione at 1000, 2000 and 3000 ppm.

Ghosh *et al.* (2002) found that Mancozeb (0.25%) and Carbendazim (0.1%) was most effective against mycelial growth and sporulation of *Alternaria alternata in vitro*.

Rao and Rao (2002) tested Captan, Carbendazim, Mancozeb and Copper oxychloride against stem blight of sesame caused by *Alternaria alternata* and observed that mancozeb was most effective in reducing disease incidence followed by Copper oxychloride and Carbendazim.

Kubota *et al.* (2003) found that application of Iprodione, Tolechophos methyl and Mancozeb, controlled the *Alternaria* sooty spot of cabbage in Japan.

Singh and Rai (2003) conducted an experiment *in vitro* to determined the effect of Indofil M-45, Indofil Z-78, Vitavax, Benlate, Thiram, Karathane, Calaxin, Ridomil, Blue copper-50, Miltox, Plant vax and Captan on the growth of *Alternaria alternata* causing *Alternaria* leaf spot of brinjal by using poisoned food technique. Indofil M-45, indofil Z-78, vitavax and kavach were at par in inhibiting *Alternaria alternata* growth (100%).

Harlapur *et al.* (2006) described that Mencozeb at 0.25% concentration gave 100% inhibition of fungus colonies in petriplate. Carboxin power @ 0.1 per cent and propiconazole @ 0.1 percent were also found effective.

Narain *et al.* (2006) evaluated the efficacy of fungicides, i.e. Bavistin (carbendazim) (0.1%), Matco (0.2%), Indofil M-45 (mancozeb + thiophanate-methyl) (0.2%), Chlorothalonil (0.2%), Roko (0.2%) and Aminocel (0.1%), against *Alternaria* leaf spot (*Alternaria brassicae*) on broccoli cv.Shadow.

Singh and Singh (2006) studied the efficacy of different fungicides i.e. hexaconazole at 1000, 500, 200, 100 and 50 ppm, Mancozeb, copper oxychloride, copper hydroxide, chlorothalonil and propineb at concentration of 2500, 2000, 1000, 500 and 250 ppm against *Alternaria alternata in vitro* by using poisoned food techniques. All fungicide significantly reduced the radial growth of the fungus. However hexaconazole was the most effective fungicide as it caused 100% growth inhibition at all tested concentrations.

Zhou *et al.* (2006) tested eight fungicides *viz.*, prochloraz, difenoconazole, carbendazim, chlorothalonil, mancozeb, copper hydroxide, iprodione and oxadixyl mancozeb to determine their toxicity against *Alternaria alternata* by using the inhibition zone method and reported that the prochloraz-manganese chloride complex and difenoconazole had the maximum toxicity.

Habib *et al.* (2007) studied the efficacy of four fungicides (Topsin M, Benlate, Dithane M-45 and Captan at three concentrations (10, 20 and 40 ppm) and it was concluded that Captan performed better against *Alternaria alternata*.

Chandra *et al.* (2009) reported that mancozeb spray showed minimum number of spots by managing *Alternaria* blight of rapeseed and mustard (*Alternaria brassicae*). They further reported that smallest size of spot was observed when leaf extracts of Eucalyptus was used for controlling *A. brassicae* causing *Alternaria* blight of rapeseed and mustard.

Nath (2012) described the use of eleven fungicides such as Blitox-50, Dithane Z-78, Thirm, Captan, Chlorothalonil, Monoceren, Aureofungin, Tilt, Ridomil MZ and Difolatan at 500µg/ml, 250µg/ml, 100µg/ml and 50µg/ml concentrations respectively. Tilt was found to be most efficient and completely inhibited the growth of fungus (*A. alternata*) at 250µg/ml and on the other hand, Blitox-50 completely inhibited the growth of the fungus at 500µg/ml. further, he described that all fungicides at 1000µg/ml. There was complete inhibition of fungal growth.

Jagana *et al.* (2013) conducted field trial and reported that Mancozeb (FS) @ 0.25 %, followed by Carbendazim (ST) @ 0.2 % + Carbendazim (FS) @ 0.2% was found to be most effective in reducing the disease intensity of *Alternaria* blight of mustard caused by *A. brassicae* with 20.9, 33.9 & 52.3% at 60, 75 & 90 DAS when

the pathogen attacked the leaves 12.0, 19.1 & 23.8 % at 75, 90 and 105 DAS in case of pods with increase in the yield (13.53 q/ha).

Neeraj and Verma (2013) reported that Mancozeb followed by Thiram, Bavistin and Iprodione were effective as seed dresser. Camalexin, brassinin, allyl -- (AllTC) and benzyl (BzITC) isothiocyanates showed antifungal effects at different developmental stages of both *A. brassicae and A. brassicicola.*

Bhargav *et al.* (2014) reported that foliar sprays of mancozeb (0.2%), Chlorothalonil (0.2%), Difenaconazole (0.1%) or Tebuconazole (0.1%) can prevent the sunflower crop from *Alternaria* blight, caused by *A. helianthi.* Propiconazole and Iprodione were also effective against the pathogen.

Harde *et al.* (2014) studied bio- efficacy of ten fungicides *in vitro* against *Alternaria brassicae.* All the fungicides tested were found fungistatic / fungicidal against the test pathogen and significantly inhibited mycelial growth of the test pathogen over untreated control. The percentage mycelial growth inhibition of the test pathogen was increased with increase in concentrations of the test fungicides. However, Iprodione 50 WP was found most effective and recorded significantly highest mean mycelial growth inhibition (100.00%). The second and third best fungicides found were Mancozeb 75 WP and Carbendazim 50 WP which recorded mean growth inhibition of 90.29 and 88.58 per cent respectively. This was followed by the fungicides, *viz.*, Chlorothalonil 75 WP (inhibition: 86.70%), Copper oxychloride 50 WP (inhibition: 86.60%), Difenconazole 25 EC (inhibition: 78.37%), Hexaconazole 5 EC (inhibition: 75.65%) and Penconazole 25 EC (inhibition: 72.18%).

Waghe *et al.* (2014) revealed the maximum inhibition of *A. helianthi* by SAAF @ 2000 ppm (90.36 %), this was followed by Mancozeb alone @ 2500 ppm (88.88 %), Propiconazole @ 1000 ppm (87.03 %), Hexaconazole @ 1000 ppm (82.59 %) and minimum inhibition recorded by Chlorothalonil @ 1000 ppm (57.03 %), this was followed by Azoxystrobin @ 500 ppm (72.96 %) as compared to (00.00 %) in untreated control.

CHAPTER-III

MATERIALS AND METHODS

MATERIALS AND METHODS

This chapter deals with the materials and methods which were used during the course of investigations and experiments.

The diseased specimens of cabbage grown in Central Farm, Orissa University of Agriculture & Technology, Bhubaneswar were collected, used for study. The symptoms were minutely observed and the disease was suspected to be *Alternaria* leaf spot. Microscopic observation of the transverse sections of affected leaves revealed the presence of characteristic mycelia, conidiophores and conidia of the fungus, *Alternaria brassicae*.

3.1 General laboratory procedures

Borosil and Corning glass wares were needed for all laboratory experiments.

3.1.1 Cleaning of glasswares

Borosil glasswares were used in laboratory experiments. They were cleaned by using standard procedures (Riker and Riker, 1936). All the glasswares were dipped overnight in solution containing 60 ml of potassium dichromate, 60 ml of conc. sulphuric acid per litre of water. Then they were washed with detergent followed by repeated rinsing with tap water. The glasswares were again rinsed with distilled water before use.

3.1.2 Sterilization

a) Sterilization of glasswares

All glasswares were sterilized in at 160-180°C temperature in hot air oven for a period of 2 hours. Before sterilization, the glasswares were wrapped with brown paper/news paper.

b) Sterilization of media

After the media were prepared, approximately 7-8ml of the medium was taken in each culture tube for the preparation of slant. The culture tubes and conical flasks containing melted agar medium were properly plugged with dry non absorbent cotton and were sterilized at 15p.s.i (121.6^0C) for 20 minutes in an autoclave. The tubes containing sterilized medium were kept in slanting position for preparation of slants.

For the preparation of petriplates, 15-20ml of sterilized medium was aseptically transferred into the sterilised petridishes and allowed to solidify. These were subsequently stored in a refrigerator at 5-8^0 C for future use.

c) Sterilization of water

For the preparation of sterilized water, desired quantity of distilled water was taken in suitable container like conical flask, plugged with non-absorbent cotton and sterilized at 15p.s.i for 20 minutes in an autoclave.

d) Sterilization of blotting paper

It was sterilized in hot air oven at 160°C for two hours by placing it inside petridish. Before keeping petridish inside oven, it was wrapped with brown paper/news paper.

e) Sterilization over flame

The inoculation needle, forecep and cork borer were sterilized by dipping them in 70% ethanol followed by flaming over spirit lamp. The slide and cover slip were placed over flame just before use.

f) Sterilization of plant material

Surface sterilization of plant material was done by cutting it into small bits (2-3mm) and then dipping these bits in (0.1%) mercuric chloride solution for 30 seconds or 0.5% sodium hypochlorite solution for 2 minutes followed by repeated rinsing with distilled water 3 to 4 times to remove the trace of chemical from the plant sample.

3.1.3 Preparation of staining solution and surface disinfectants

a) Preparation of cotton blue

For preparation 1% of cotton blue, 1g cotton blue crystals were dissolved in 100 ml of lactophenol.

b) Preparation of lacto phenol

Chemicals:

Lactic acid	100 ml
Phenol	100 ml
Glycerin	100 ml
Distilled water	100 ml

Lacto phenol solution was made by dissolving phenol in water without heat to prevent oxidation. Then glycerine was added to it followed by lactic acid.

c) **Preparation of mercuric chloride**

Stock solution:

Mercuric chloride	20 ml
Conc. Hydrochloric acid	100 ml

Disinfecting solution:

Stock solution	5 ml
Distilled water	995 ml

For preparation of stock solution, 20g of mercuric chloride was dissolved in 100 ml of conc. Hydrochloric acid. At the time of use 5 ml of stock solution was dissolved in 995 ml of distilled water.

d) **Preparation of 4% formalin solution**

For preparation of 4% formalin solution, 100 ml of commercial formaldehyde was added in 900 ml of distilled water.

Maintenance of aseptic condition

To avoid contamination, all the operations were carried out inside an inoculation chamber under the laminar air flow in aseptic condition.

3.2 Collection of the disease sample

Disease samples were collected from nearby farms and from central farm of Orissa University of Agriculture and Technology Bhubaneswar. Each sample was brought to the laboratory separately in polythene bags and being washed with distilled water. Affected portions of the leaves were teased and examined under microscope which revealed the presence of characteristic mycelia, conidiophores and conidia of the fungus, *A. Brassicae*.

3.3 Isolation of the pathogen

The infected leaves showing typical disease symptoms were cut into small bits containing infected portions well as healthy portion. These bits were sterilized with 0.5% sodium hypochlorite for 2 minutes or 0.1 % mercuric chloride for 30 seconds and then washed serially 3 times in sterilized distilled water for 2 minutes each to

remove the traces of surface disinfectants. Then the bits were transferred to sterilized petriplates (four bits at four corners) containing potato dextrose agar by sterilized forcep. The petiplates were kept for incubation at room temperature at 27±1°C for 7 days and the growth of the fungi on the petriplates was recorded on the 7th day after incubation. Further purification was done in potato dextrose agar whenever necessary.

3.3.1 Purification and identification of fungi

Each isolate of fungus maintained on PDA slants, were transferred to agar plates. Hyphae from the periphery of young colonies were carefully examined 'and transferred to PDA slants. This process was repeated for 2-3 times till the concerned fungus was found to be free from other fungi and bacteria. Pure culture of fungus was obtained through 'single spore' and 'hyphal tip' methods. The characteristics of the fungal colony on PDA and the details of their morphology were recorded for each isolate. Each isolate was taxonomically identified with the help of available cultures and literature.

3.3.2 Single spore isolation

A spore suspension was prepared in sterile water and spore contents were ascertained by examining a drop of suspension under the microscope. Culture tubes containing 10ml of sterile water agar were melted over water bath and temperature was allowed to come down to 40°C. A loop full of spore suspension from this tube was again transferred to second culture tube containing melted agar. Likewise a series of dilution of spores in the medium were prepared which in turn poured in to the sterilized petriplates one after another. All petriplates were incubated in an incubator at 28±1°C.

After 24 hours of incubation, the petriplates were examined in an inverted position and germinating spores were located and marked with the help of glass marking pencil. A small amount of medium in the marked area containing germinating spores were transferred by means of a sterilized inoculating needle to the middle of potato dextrose agar medium slant under aseptic condition. The slants with single germinating spore were incubated in a BOD incubator at 28±1°C. After 2-3 days, the growth of the fungus was observed in the slant. Thus a pure culture was obtained.

3.3.3 Hyphal tip culture

The fungus was grown in a sterilised petridish containing potato dextrose agar medium. As isolated, hyphal tip was located under the microscope and marked with the help of a sharp glass marking pencil. The tip was carefully lifted up and transferred by sterilized inoculating needle to a potato dextrose agar slant at room temperature. After 2-3 days, the growth of the fungus was observed in the culture tube and thus a pure culture of the fungus was obtained. After getting the fungus in pure culture, it was maintained in potato dextrose agar medium and sub cultured in 2 weeks intervals.

3.3.4 Identification of the culture

The culture obtained was compared with the original description of the fungus for morphological characters.

A bit of fungal hypha was taken from 15 days old culture on a slide and teased gently by the help of two sterilized pricking needle. Then it was stained with lactophenol blue, covered with glass cover slips and examined under microscopes to study the morphological characters like its mycelia growth, colony characteristics like its colour, texture, lustre and growth habit etc., size, shape, colour, septations etc. of the conidia with the help of microscope. Microphotograph of the mycelium and conidia was taken and measurement of the conidia was done with the help the computer generated micrometer.

3.3.5 Maintenance of the culture

The fungus was sub cultured on the PDA slants and allowed to grow at room temperature for 15 days. Then the slants were preserved in refrigerators at 4°C and sub-cultured once in 30 days.

3.3.6 Proving Pathogenicity

Pathogenicity was proved on local variety of cabbage plant. The healthy plants were raised in earthen pots. Sixty days old plants were sprayed with distilled water then they were covered with polythene bags for 24 hours. Two methods were used for inoculation.

1. The leaves of the plants were injured slightly by pricking with the help of the sterilized needle. The leaves were then sprayed with suspension containing mycelial bits and spores of the fungus which was prepared in sterilized water. The control plants were sprayed with only sterilized distilled water. After spraying, all the plants were covered with polythene bags and kept inside a glass house at 25°C and 95% RH.

   Observation on occurrence of disease symptom was recorded regularly.
2. Some places on the leaf lamina were selected and they were marked by ink. Then the marked portions were smeared with mycelial bits by using a cotton plug. Similarly control plants were smeared with distilled water for comparison. The symptom appeared after 9 days of inoculation and re isolation was made from such spots. The obtained isolate was compared with the original culture for confirmation.

3.4 Morphological studies

A mycelial tip was taken from the culture. It was teased upon a glass slide and a drop of cotton blue lactophenol was added upon that and was covered by a cover slip. The slide was observed under compound microscope. The typical characters of the fungus were observed and they were compared with the references. The spore was measured under low power objectives as well as under high power objective and the microphotographs were taken.

3.5 Spore germination studies

Spore suspension was made in sterilized water. A drop of spore suspension was taken upon a clean slide. Then the slide was placed in petridish lined with wetted blotting paper to provide high humidity. It was kept for 24 hours. Then the type of germination was studied under microscope.

3.6 Cultural studies

3.6.1 Growth on solid media

The growth characters of *Alternaria brassicae* were studied on 9 different solid media viz. Potato dextrose agar, Host extract agar, Carrot root extract agar, Oat meal agar, Malt extract agar, Czapek's (Dox) agar, Richard's agar, Sabouraud dextrose agar and Asthana & Hawker's agar. All the media were sterilized at 15 psi

for 20 minutes. After sterilization, each medium was poured into 90 mm petridishes. Each treatment was replicated thrice. After pouring they were allowed to cool down. Then 5 mm mycelia disc of culture was inoculated into each plate and incubated at room temperature i.e.28±1°C. Colony diameter was recorded by averaging linear growth of the colony in three directions for each plate at 9 days of inoculation with the help of fine transparent plastic scale in millimetres. The colour of the fungal colony, surface elevation and sporulation were also recorded. The recorded data were analysed statistically. The composition of each medium given below. The preparation of various media was done following the procedure given by Ainsworth (1971) and Tuite (1969).

Potato dextrose agar

Potato Dextrose Agar (PDA) medium was prepared by following standard procedure postulated by Riker and Riker (1936) followed with slight modifications wherever necessary.

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

Two hundred gram of peeled and sliced potato was boiled in a container with 500 ml of water for some time until it was soften, but not over cooked. The extract was filtered through a piece of muslin cloth. Simultaneously, agar was melted in 500 ml of water in another container. The potato decoction and the melted agar were mixed together to which dextrose was added and mixed thoroughly in another container with constant stirring and the volume was made up to 1000 ml. Streptomycin sulphate was added to the medium @ 0.75g per 1000 ml before autoclaving for suppression of bacterial contamination. The prepared potato dextrose agar medium was kept in suitable flasks or tubes, plugged and sterilized in autoclave at 15 psi for 20 minutes.

Host extract agar

Healthy cabbage leaves (young) - 200g

Agar-agar -20g

Distilled water - 1000ml

In Host extract agar medium, 200g of leaves were washed, crushed with the help of mortar and pestle and boiled in 500ml of distilled water for half an hour. The extract was taken out through muslin cloth. To this, 20g agar-agar was added and boiled. Final volume was made up to 1000ml. Streptomycin sulphate was added to the medium @ 0.75g per 1000 ml before autoclaving for suppression of bacterial contamination. Then the medium was sterilized in autoclave at 15 psi for 20 minutes.

Carrot root extract agar

Peeled and sliced carrot	-250g
Agar-agar	-20g
Distilled water	-1000ml

The carrots were washed, crushed with the help of mortar and pestle and boiled in 500ml of distilled water for half an hour. The extract was collected by filtering through a muslin cloth. Agar-agar was melted separately in 500 ml of distilled water. The carrot extract was mixed in the molten agar and 20g of dextrose was added to the mixture. The volume was made up to 1000ml with distilled water. Streptomycin sulphate was added to the medium @ 0.75g per 1000 ml before autoclaving for suppression of bacterial contamination. The prepared carrot root extract agar medium was kept in suitable flasks or tubes, plugged and sterilized in autoclave at 15 psi for 20 minutes.

Oat meal agar:

Oat flakes	60 g
Agar-agar	20 g
Distilled water	1000 ml

Oat flakes were boiled in 500 ml of distilled water for 20 minutes. Then the extract was collected by filtering through muslin cloth. Agra-agar was melted separately in 500 ml of water by boiling. Then the two mixtures were mixed thoroughly and volume was adjusted up to 1000ml. Then 0.75 g of streptomycin sulphate was added into it to avoid bacterial contamination. Then the medium was sterilized in autoclave at 15 psi for 20 minutes.

Malt extract agar:

Malt extracts	25 g
Agar-agar	20 g
Distilled water	1000 ml

Malt extract was dissolved in 500 ml of distilled water. Agar-agar was melted separately in 500 ml of water by boiling. Then malt extract was added in the melted agar and thoroughly mixed. 0.8 g of streptomycin was added into it to avoid bacterial contamination. Then the medium was sterilized in autoclave at 15 psi for 20 minutes.

Czapek's (Dox) agar

Sucrose	30 g
Sodium nitrate	2 g
Potassium dihydrogen phosphate	1 g
Magnesium sulphate	2.5 g
Potassium chloride	0.5 g
Ferrous sulphate	0.01 g
Agar-agar	20 g
Distilled water	1000 ml

All the ingredients were mixed in 500 ml of distilled water. Agar-agar was melted separately in 500 ml water by boiling. Then two mixtures were mixed and streptomycin sulphate 0.75 g was added for avoidance of bacterial contamination. The medium was sterilized in 15 psi for 20 minutes in autoclave.

Richard's agar:

Sucrose	50 g
Potassium nitrate	10 g
Potassium dihydrogen phosphate	5 g
Magnesium sulphate	2.5 g
Ferric chloride	0.02g
Agar-agar	20 g
Distilled water	1000 ml

All the ingredients were mixed in 500 ml of distilled water. Agar-agar was melted separately in 500 ml water by boiling. Then two mixtures were mixed and streptomycin 0.8 g was added for avoidance of bacterial contamination. The medium was sterilized at 15 psi for 20 minutes in autoclave.

Sabouraud dextrose agar

Peptone	10 g
Dextrose	20g
Agar-agar	20 g
Distilled water	1000 ml

All the ingredients were mixed in 500 ml of distilled water. Agar-agar was melted separately in 500 ml water by boiling. Then two mixtures were mixed and streptomycin sulphate 0.75 g was added for avoidance of bacterial contamination. The medium was sterilized in 15 psi for 20 minutes in autoclave.

Asthana & Hawker's agar

Glucose	5g
Potassium dihydrogen phosphate	1.75g
Potassium nitrate	3.5g
Magnesium sulphate	0.75g
Agar-agar	20g
Distilled water	1000ml

All the ingredients were mixed in 500 ml of distilled water. Agar-agar was melted separately in 500 ml water by boiling. Then two mixtures were mixed and streptomycin 0.8 g was added for avoidance of bacterial contamination. The medium was sterilized at 15 psi for 20 minutes in autoclave.

3.6.2 Growth characters on liquid media

The composition and preparation of different liquid media were same as that of solid media except that here agar-agar was not added. Thirty ml from each media were taken in a 100ml conical flask and were sterilized in the autoclave at 15 lb pressure for 20

minutes. After the media are cooled down, a 5mm disc from an actively growing zone of 10 days old culture was placed upside down at the surface of the liquid media aseptically and incubated at 27±1^{0}C.Each treatment was replicated thrice.

When the maximum growth was attained in any one of the media tested photographs were taken to show the growth behaviour of the pathogen. Filtering was done with the help of a pre weighed Whatman No 1 filter paper in order to get the mycelia mats. The mycelial mats along with the filter papers were dried in a hot air oven at 60^{0}C for one hour. Then the weights of the filter papers along with the mycelia mats were taken. Weight of only mycelia mat was found out by the formula.

Wt. of mycelia mat = Wt. of filter paper along with mycelia mat – Wt. of filter paper

3.7 Nutritional studies

3.7.1 Carbon Utilization

The carbon requirement of the fungus was studied in Richard's broth. The amounts of carbon compound used were calculated according to their molecular weight so as to provide the equivalent amount of carbon as sucrose present in the basal medium. Carbon sources used in the experiment were glucose, dextrose, sucrose, L-asparagine, glycine, starch, xylose and maltose. Each treatment was replicated thrice. One set was maintained as control without adding any carbon source. All the treatments were sterilized in autoclave at 15 psi for 20 minutes. The flasks were inoculated with 5 mm mycelia disc of culture and incubated at room temperature for 10 days. After that the mycelial mats were harvested and dry weight of each mycelia mat was recorded. The data were analysed statistically.

3.7.2 Nitrogen Utilization

The nitrogen requirement of the fungus was studied in Richard's broth. The amount of nitrogen compounds were calculated according to their molecular weight so as to provide the equivalent amount of nitrogen as Potassium nitrate in the Sodium nitrate, Ammonium oxalate, L-asparagine, Ammonium carbonate and Ammonium per sulphate. Each treatment was replicated thrice. One set was maintained as control

without adding any nitrogen source. All the treatments were sterilized in autoclave at 15 psi for 20 minutes. The flasks were inoculated with 5 mm mycelia disc of culture and incubated at room temperature for 10 days. After that the mycelial mats were harvested and dry weights of each mycelial mat were recorded. The data were analysed statistically.

3.8 Physiological studies

3.8.1 Hydrogen ion concentration (pH)

The pH requirement of the fungus was studied in potato dextrose broth. One hundred ml capacity conical flasks containing 30 ml medium each were taken and pH of each medium was adjusted to different pH levels (3, 4, 5,6,7,8 and 9) with the help of a digital pH meter by adding 0.1N Hcl and / or 0.1N NaOH. These were then sterilized at 15 psi for 15 minutes in an autoclave. The pH level of medium after sterilization was found unaltered. A mycelia disc of 5 mm diameter was taken from 10 days old culture of *A. Brassicae* and was incubated at room temperature. Each treatment was replicated thrice. The dry weight of the mycelial mat was taken after 15 days of incubation. Then data were analysed statistically.

3.8.2 Effect of light on the growth of test fungus

The effect of light on growth of *A. brassicae* was studied on potato dextrose agar by exposing the pure cultures to 8 h dark 16 h light; 8 h light 16 h dark, 12 h dark 12 h light and 24 h dark and 24 h light. The inoculation of the test fungus to petriplates containing potato dextrose agar were done as explained earlier. The plates were incubated at 25± 1 °C for 10 days. Observations on colony diameter were recorded as described earlier and the data were analyzed statistically.

3.9 Management studies

3.9.1 *In vitro* evaluation of botanicals

Use of botanicals to manage the pathogen is safe, eco-friendly and economical. In the present study, the following botanicals (Table-1) were selected to observe their effect upon the fungus.

Table -1 List of plant extracts in *in vitro* evaluation of test fungus

Sl. No.	Common Name	Scientific name	Family	Plant part used
1	Milkweed	*Calotropis procera*	Apocynaceae	Leaf
2	Deodar	*Polyalthia longifolia*	Annonaceae	Leaf
3	Karanj	*Pongamia pinnata*	Fabaceae	Leaf
4	Datura	*Datura stramonium*	Solanaceae	Leaf
5	Lantana	*Lantana camara*	Verbenaceae	Leaf
6	Neem	*Azadirachta indica*	Meliaceae	Leaf
7	Onion	*Allium cepa*	Amaryllidaceae	Bulb
8	Eucalyptus	*Eucalyptus globules*	Myrtaceae	Leaf
9	Bisalyakarani	*Tridex procumbens*	Asteraceae	Leaf
10	Tulsi	*Ocimum sanctum*	Lamiaceae	Leaf
11	Garlic	*Allium sativum*	Amaryllidaceae	Bulb
	Control			

Preparation of crude extract of plants

The fresh plant parts i.e. bulbs, leaves and rhizomes were collected from the field and washed first in tap water and then distilled water. After that they were air dried for few minutes. Hundred gram of each sample was taken with equal amount of water (1:1 w/v) and grinded by using mortar and pestle (Wokocha and Okereke, 2005). The extract was filtered through two layers of muslin cloth followed by Zeitz filter to free from bacterial contamination. This extract was taken as stock solution.

Poisoned food technique

The bio-efficiency of the botanicals against the fungus evaluated by poisoned food technique (Nene and Thapliyal, 1973). Ten and 20 ml of stock solution was mixed with 90 and 80ml of sterilized molten potato dextrose agar medium respectively so as to get 10% & 20% concentration. The medium was thoroughly shaken for uniform mixing of extract.

Twenty ml of medium of each concentration poured into sterilised petridishes. Each treatment was replicated thrice. The medium was allowed to cool. Then 5 mm mycelia disc was taken from periphery of actively growing culture and inoculated at the middle of the petriplate. One set of control was maintained without adding any plant extract. After inoculation, the plates were incubated at room temperature. The observation was taken when the radial growth of the control plate was maximum. The growth of the other plates were compared with the control plates. Observation was taken. The efficacy of the plant extracts was expressed in percentage inhibition of the

radial growth over the control. The formula for the calculation given by Vincent (1947) as follows.

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

I = Percent inhibition of mycelium

C = Radial growth of mycelium in control

T = Radial growth of mycelium in treatment

3.9.2 *In vitro* evaluation of oils

Pre-standardizing experiment was carried out with different concentrations (0.5, 0.75 and 1.0%) for all the plant oils and finalized the concentration of oil for testing the efficacy. The appropriate concentration of oil after emulsifying with teepol at one ml per liter mixed with sterilized potato dextrose agar and thoroughly mixed just before plating so as to get the specified concentration of the plant oils. Twenty ml of this mixture was poured in to a sterilized petridish (90 mm in diameter) in three replications and allowed to solidify. A 5 mm culture disc of the pathogen was taken and placed on the centre of the medium. The plates were incubated at room temperature at 27±1°C. The radial growth of the colony in each plate was measured when the control plate showed full growth. The efficacy of the oils was expressed in percentage inhibition of the radial growth over the control. The formula for the calculation was

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

I = Percent inhibition

C = Radial growth in control

T = Radial growth in treatment

3.9.3 *In vitro* evalution of biocontrol agents:

The efficiency of biocontrol agents against the fungus *A brassicae* was tested by dual culture method. Biocontrol agents like *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas flourescens*, *Bacillus subtilis* were tested against the fungus. The fungal antagonists were grown in potato dextrose agar and the bacterial antagonists were grown in nutrient agar medium to get fresh and active culture for the experiment.

Dual culture method:

About 20 ml of sterilized medium (Potato dextrose agar for fungus and nutrient agar medium for bacteria) was taken in sterilized petridish and allowed to cool. Then the fungal mycelial disc was transferred to petriplate and the mycelial disc of fungal antagonist was transferred to the opposite end of the petriplate. In case of bacterial antagonists, the bacterium was streaked at the centre of the petriplate and two mycelial discs were placed at opposite ends. Each treatment was replicated four times. The inoculated plates were incubated at room temperature for five days. After five days the observation was taken. The percent of inhibition was worked out according to the formula given by Vincent (1947). The data was analysed statistically.

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

I = Percent inhibition

C = radial growth in control

T = Radial growth in treatment

3.9.4. *In vitro* evaluation of fungicides:

The bio-efficiency of eleven fungicides were tested against the fungus *Alternaria brassicae* at their recommended dose. These fungicides were

Table-2 List of different fungicides tested for efficacy

Treatment	Chemical name	Trade name	Recommended dose (%)
T1	Carbendazim12%WP+Mancozeb63%WP	Saaf	0.2
T2	Fenamidione10%WP+Mancozeb	Sectin	0.1
T3	Chlorothalonil 75%WP	Kavach	0.2
T4	Difenconazole 25%EC	Score	0.05
T5	Copper hydroxide 77%WP	Kocide	0.1
T6	Thiophanate Methyl 70%WP	Roko	0.15
T7	Azoxystrobin 23% EC	Amistar	0.1
T8	Copper oxychloride	Nagcoper	0.3
T9	Propiconazole 25% EC	Tilt	0.15
T10	Control		

The bio-efficiency of the fungicides were evaluated by poisoned food technique (Nene and Thapliyal, 1973). The required concentrations of chemicals were prepared and incorporated into sterilized, cooled potato dextrose agar medium.

Twenty ml of poisoned medium was poured in each petridish. Each treatment was replicated thrice. One set of control was maintained without addition of any fungicides. 5 mm mycelial disc of the culture was transferred to each plate aseptically. Then the plates were incubated at room temperature for 15 days. When there was maximum radial growth in the control plate, the observation was taken. The data were analysed. The efficacy of the fungicides was expressed as percentage of inhibition of mycelia growth over control. The percentage of inhibition of mycelium was calculated by the formula (Vincent, 1947).

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

I = Percent inhibition

C = radial growth in control

T = Radial growth in treatment

CHAPTER-IV

RESULTS

RESULTS

4 Experimental results

The experimental results of the studies on *Alternaria* blight of cabbage conducted under protected cultivation and its management are presented below.

4.1. Study on symptoms of *Alternaria* blight of cabbage

During investigation, the disease symptoms were well studied. It was found that all stages of crop growth were affected by *Alternaria* blight. At nursery stage the infected seedlings develop spots on leaves which results into stunting and sometime into damping off. As the disease progress, it spreads to all aerial parts of plant. Initially minute yellow specks were found on leaves and stem. These spots gradually darken and enlarge into circular, tan to dark coloured concentric rings gave the appearance of target board and yellow halo was seen surrounding the lesion. As the spots become older it changed to tan, brown or black in colour, papery in texture and finally falls off giving the appearance of shot hole (Figure-1-3).

4.2 Isolation and identification of the pathogen

Infected fresh leaf samples were collected and thin transverse section of the affected parts were prepared and examined under microscope to observe the morphological characters of the fungus. From the fresh affected leaf sample, the fungal pathogen *Alternaria* sp. could be observed consistently on microscopic study.

The pure culture of the fungus was also in slants after isolation from the disease samples and the cultural characteristics were observed.

4.2.1 Study of cultural characteristics

The fungus in pure culture produced colonies with profuse cottony mycelium, cobweb like, whitish to light grey in colour which turned dark with age. Sometimes concentric ring of conidial patches which were tea green to deep olive. The submerged mycelium has a radiate to curly growth and colourless to deep greyish olive to dark olive. (Figure: 4- 6).

Fig.1 Initial symptom of leaf blight on cabbage leaf

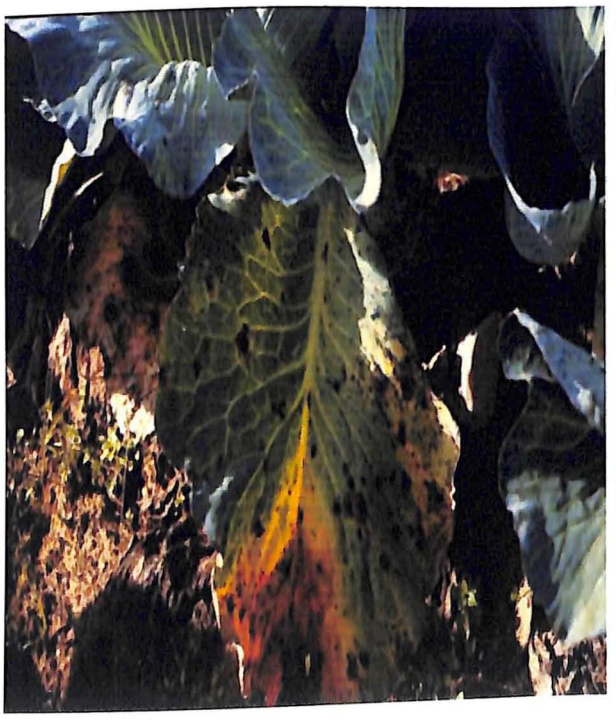

Fig. 2 Later stage of leaf symptom

Fig.3 Leaf spot enlarged into concentric rings

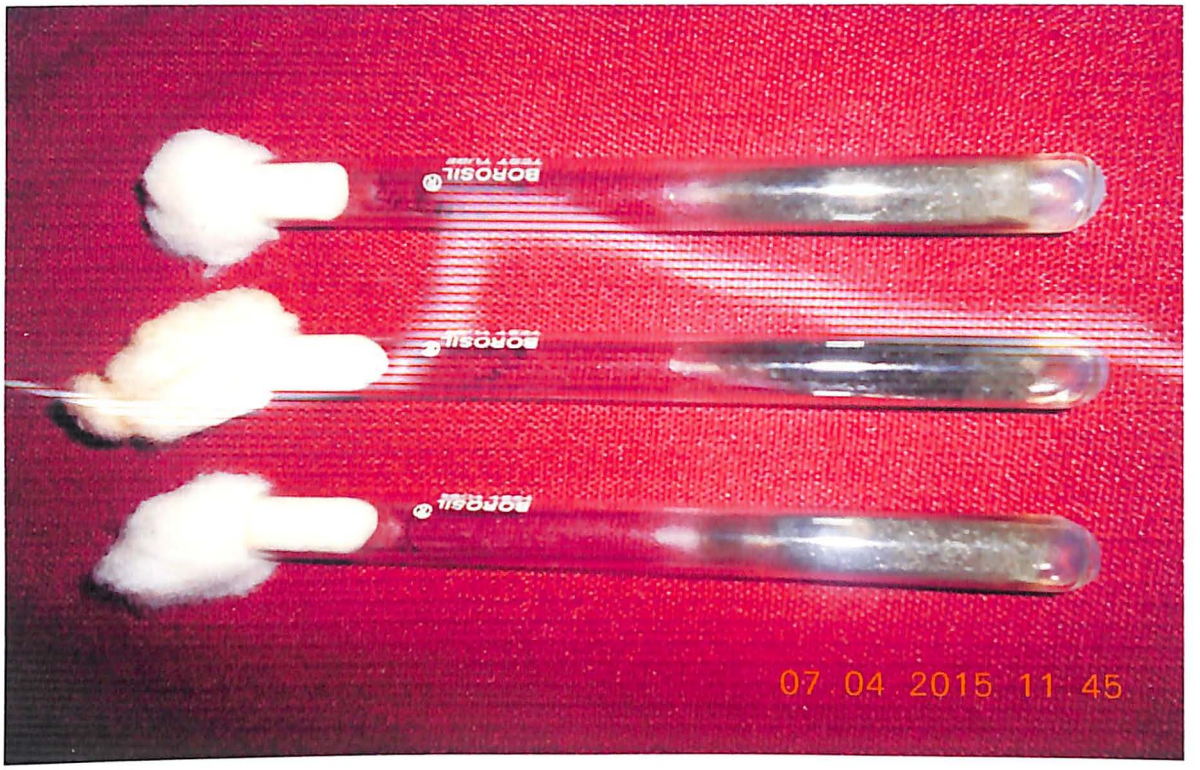

Fig. 4 Pure culture in slants

Fig. 5 Seven days old pure culture of *Alternaria brassicae*

Fig. 6 Fifteen days old pure culture of *Alternaria brassicae*

4.2.2 Study of morphological and taxonomic characteristics

Microscopic examination revealed the presence of brownish black, obclavate with 4-11 transverse and 0-4 longitudinal septate conidia with a size of 28.85-67.28 × 11.39-13.91 µm with the average of 41.90 × 12.94 µm including the length of the beak. Borne singly or sparingly in chains of 2-4. Conidiophores of the fungus were long and distinctly geniculate, arise in fascicles, dark brown, un-branched, 1-5 septate and erect. (Figure:7 and 8).

The taxonomic position of *Alternaria brassicae* is as followes:

Kingdom	Fungi
Phylum	Ascomycota
Class	Dothideomycetes
Subclass	Pleosporomycetidae
Order	Pleosporales
Family	Pleosporaceae
Genus	*Alternaria*
Species	*brassicae*

4.3 Pathogenicity test

The pathogenicity of the fungus was proved on potted cabbage plants following the postulations demonstrated by Robert Koch (1882) as per the procedure described in materials and methods. Symptoms were observed after 7 days where the control plants did not show any symptoms (Figure: 9 - 12).

The symptoms produced by the fungus as well as its cultural, morphological and taxonomic characteristics were studied in detail and compared with the symptoms occurred in nature and fungus isolated originally from the diseased samples. In both the cases it was found similar and identified as *Alternaria brassicae.*

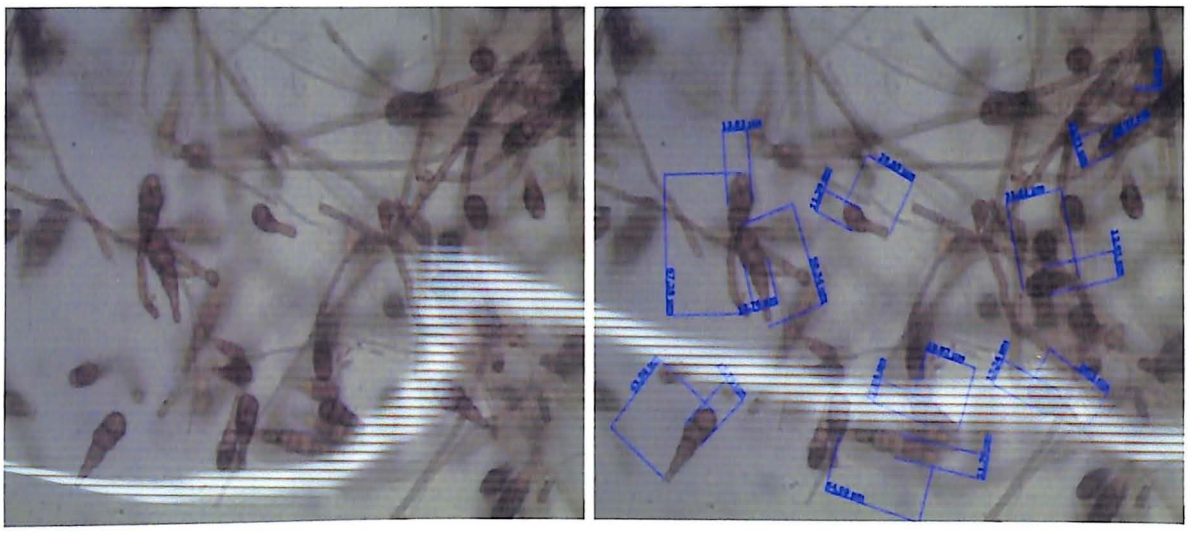

Fig.7 Micro photograph showing conidia and conidiophores and their measurements

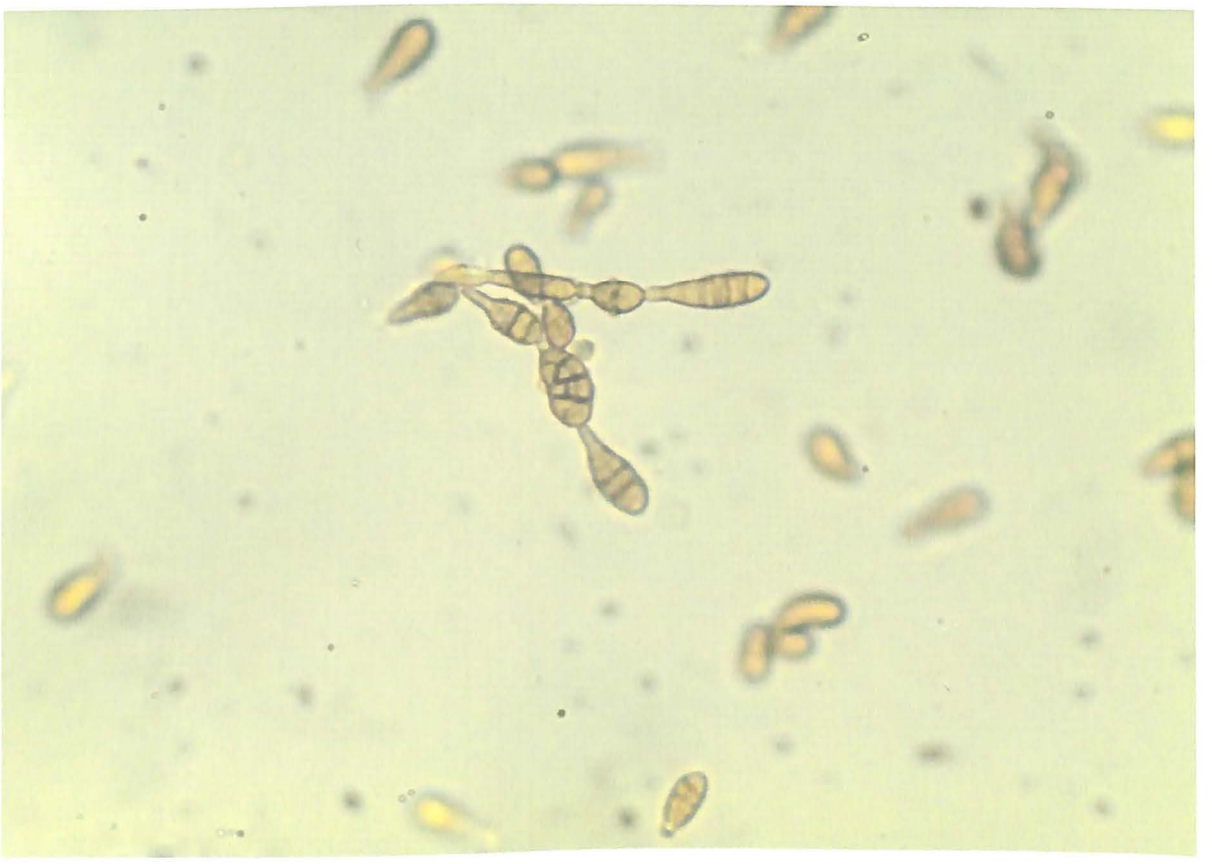

Fig. 8 Microphotograph showing conidia in chains

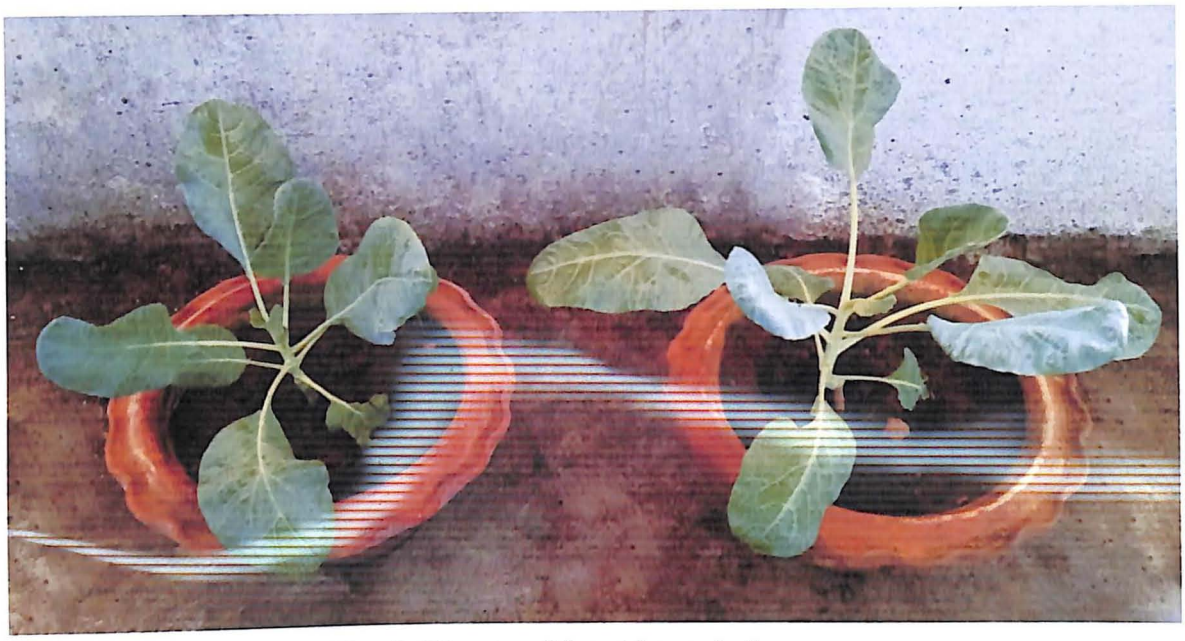

Fig. 9 Plants without inoculation

Fig. 10 Inoculated plants

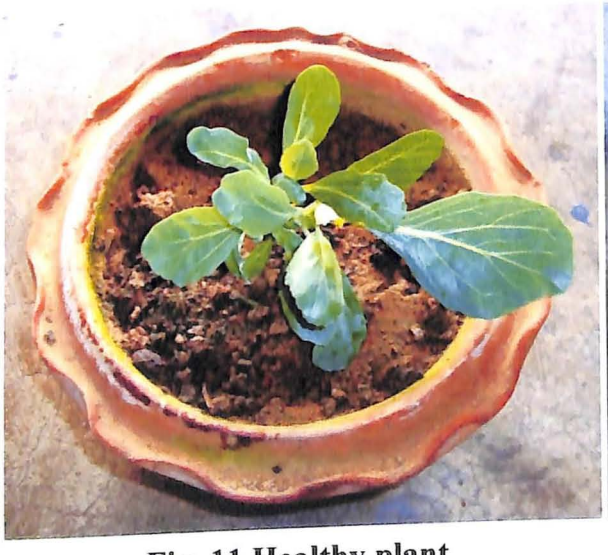

Fig. 11 Healthy plant

Fig. 12 Diseased plant

4.4 Cultural studies

4.4.1. Growth and sporulation on different solid media

In order to find out a suitable medium for growth and sporulation of the test fungus, nine different solid media of synthetic, semi-synthetic and natural were tried as per the procedure described under "Materials and Methods".

Observations on radial growth of the fungal colony and sporulation were recorded after ten days of inoculation. The data obtained have been presented in Table 3 and depicted in Figure 13.

Data in the table revealed that Potato dextrose agar medium supported significantly the maximum radial growth (85.89mm) of the test fungus followed by Sabouraud dextrose agar medium (76.11mm) and malt extract agar (71.63mm). The other nutrient media namely Richard's agar, Host extract agar, Oat meal agar, Carrot root extract agar, Czapek's agar, Asthana & Hawker's agar were found inferior in respect of radial growth of fungus with the mean colony diameter of 68.11, 65.28, 64.14, 58.4, 56.99 and 54.48mm respectively.

Similarly maximum sporulation was observed in Potato dextrose agar medium. Least sporulation was recorded in case of Carrot root extract agar, Czapek's agar Asthana & Hawker's agar medium.

Table 3 Effect of different solid media on the growth of *Alternaria brassicae*

Sl. No.	Treatments (Solid media)	Mean colony diameter (mm)*	Sporulation
1	Potato dextrose agar	85.89	++++
2	Host extract agar	65.28	++
3	Carrot root extract agar	58.4	+
4	Oat meal agar	64.14	++
5	Malt extract agar	71.63	+++
6	Czapek's Dox agar	56.99	+
7	Richard's agar	68.11	+++
8	Sabouraud dextrose agar	76.11	+++
9	Asthana & Hawker's agar	54.48	+
S.Em±		1.219	
CD (5%)		3.62	
CV (%)		3.16	

No sporulation; + low; ++ moderate; +++ profuse; ++++ abundant * Mean of three replications

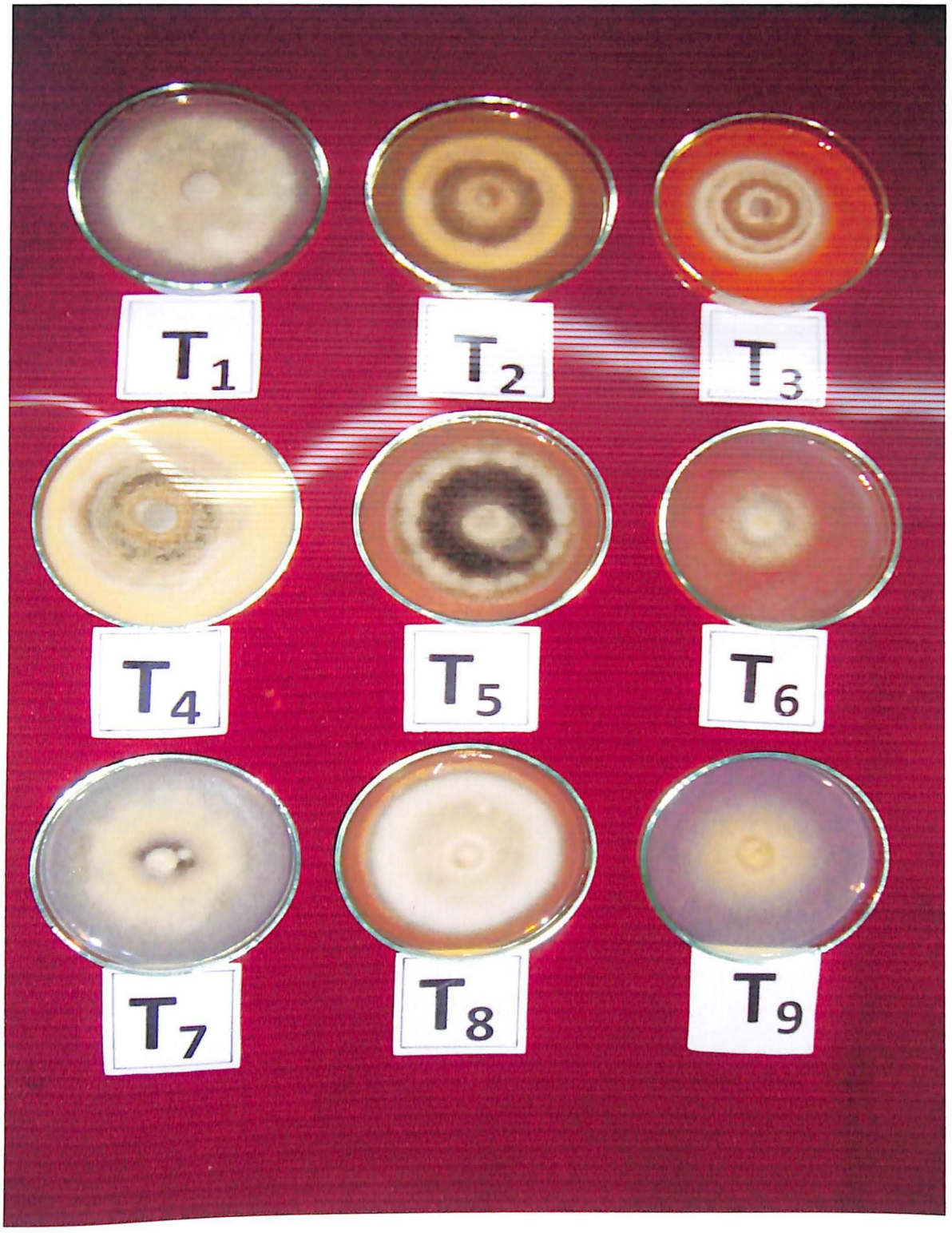

Fig. 13 Growth of *Alternaria brassicae* in different solid media

T_1 : Potata dextrose agar
T_2 : Host extract agar
T_3 : Carrot root extract agar
T_4 : Oat meal agar
T_5 : Malt extract agar
T_6 : Czapek's Dox agar
T_7 : Richard's agar
T_8 : Sabouraud dextrose agar
T_9 : Asthana & Hawker's agar

4.4.2. Growth on different broth media

In order to study the dry weight growth of the test fungus, the same was grown on nine different liquid media *i.e.* broth media (Figure 14) including synthetic, semi-synthetic and natural media as described under the "Materials and Methods" and the data recorded are presented in the Table 4.

Data in the table revealed that Richard's broth medium supported maximum dry weight growth (653.41mg) of the test fungus followed by Oat meal broth (640mg) and Czapek's broth (343.33mg). Lowest dry mycelial weight was obtained in case of Asthana & Hawker's broth (96.67mg) followed by host extract broth (136.67mg).

Table-4 Effect of different liquid media on the growth of *Alternaria brassicae*

Sl. No.	Treatments (Liquid media)	Mean dry mycelia weight(mg)
1	Potato dextrose broth	280
2	Host extract broth	136.67
3	Carrot root extract broth	296.95
4	Oat meal broth	640
5	Malt extract broth	216.77
6	Czapek's Dox broth	343.33
7	Richard's broth	653.41
8	Sabouraud dextrose broth	233.33
9	Asthana & Hawker's broth	96.67
SEm±		21.961
CD (5%)		65.24
CV%		11.82

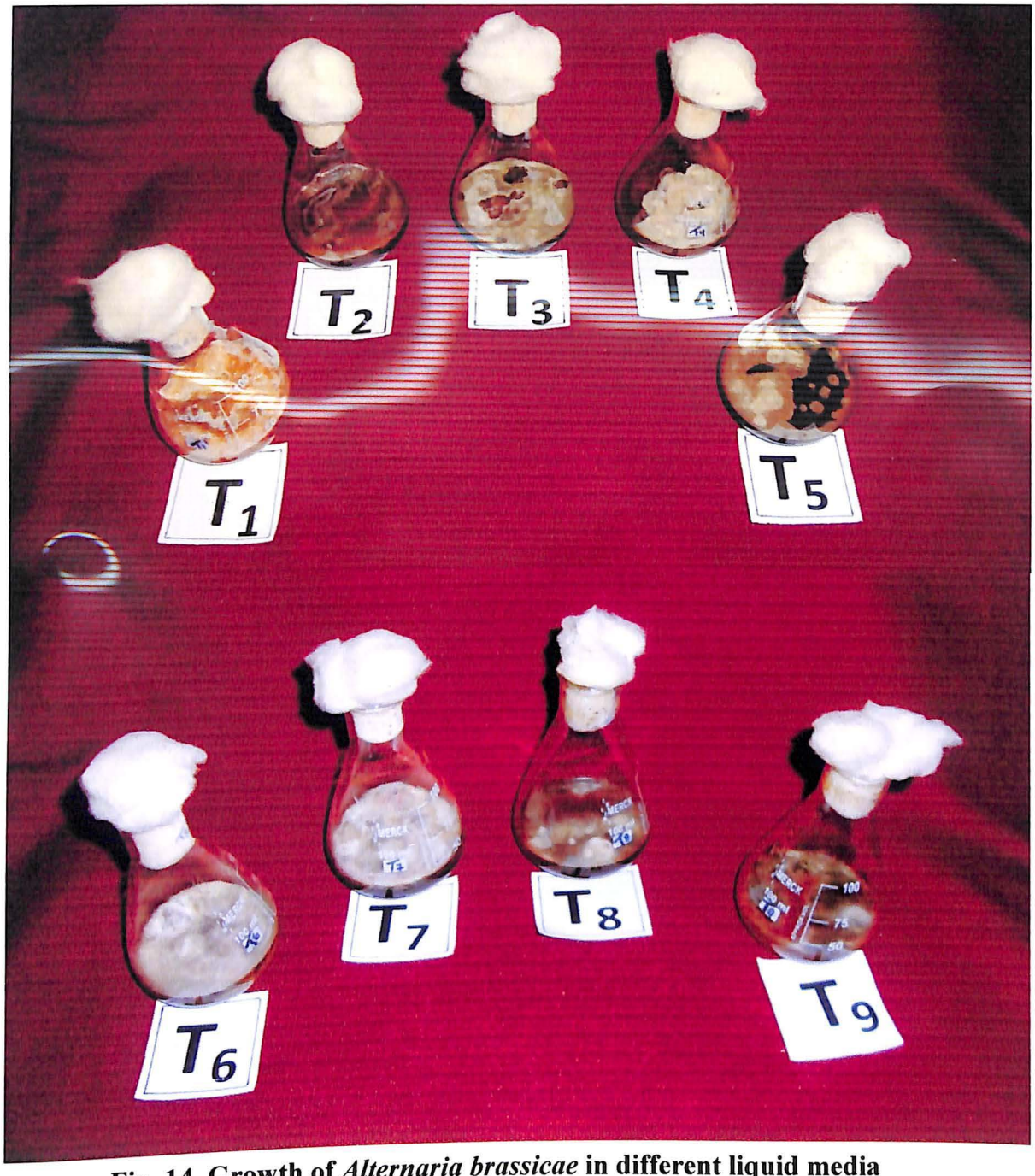

Fig. 14 Growth of *Alternaria brassicae* in different liquid media

T1 : Potata dextrose broth
T_2 : Host extract broth
T_3 : Carrot root extract broth
T_4 : Oat meal broth
T_5 : Malt extract broth
T_6 : Czapek's Dox broth
T_7 : Richard's broth
T_8 : Sabouraud dextrose broth
T_9 : Asthana & Hawker's broth

were inferior in supporting the vegetative growth of the test fungus yielding the corresponding mean dry weight of 186.67 mg & 140.12 mg and recorded poor on Richard's broth excluding the carbon source (control) with a dry mycelial weight of 23.33 mg. However, rate of sporulation was maximum in sucrose supplemented medium and glycin was inferior for spore production.

4.5.2. Effect of nitrogen Sources on the growth and sporulation of *Alternaria brassicae*.

Six nitrogen sources along with control evaluated to assess their performance in terms of the extent of vegetative growth of the test fungus. Analysis of data revealed that L-asparagine as a nitrogen source, supported maximum dry mycelial weight growth of test fungus (676.67mg) followed by ammonium oxalate (600.12mg), sodium nitrate (516.67mg) and potassium nitrate (510.23mg). Further, ammonium carbonate, ammonium persulphate and Richard's medium without any nitrogen source (control) was found to be inferior in expressing the mycelial growth of test fungus with the mean dry mycelial weight of giving the mean dry mycelial weight of 243.33, 176.85 and 146.67mg respectively. However, rate of sporulation was maximum in asparagine supplemented medium and ammonium persulphate was inferior for production of spores (Table-6 and figure-16). The table below shows its effect on growth and sporulation of test fungus.

Table 6 Effect of different nitrogen sources on the growth and sporulation of *Alternaria brassicae*

Sl. No.	Nitrogen sources	Mean dry mycelia weight (mg)	Sporulation
1	Potassium nitrate	510.23	+++
2	Sodium nitrate	516.67	+++
3	Ammonium oxalate	600.12	+++
4	L-Asparagine	676.67	++++
5	Ammonium carbonate	243.33	+
6	Ammonium persulphate	176.85	+
7	Control	146.67	-
	SEm±	26.073	
	CD (5%)	79.07	
	CV %	11.01	

No sporulation; + low; ++ moderate; +++ profuse; ++++ abundant

* Mean of three replications

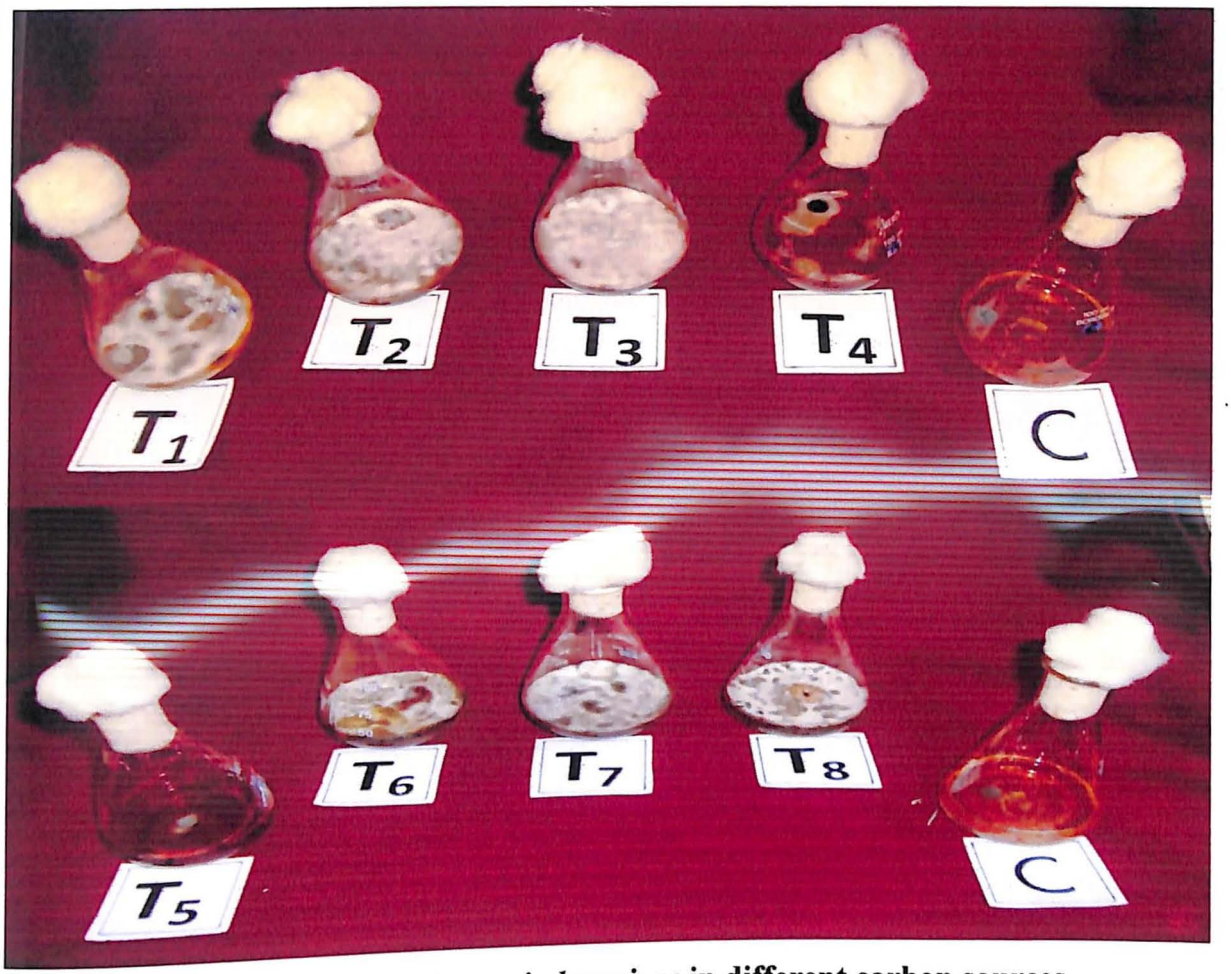

Fig. 15 Growth of *Alternaria brassicae* in different carbon sources

T_1 : Glucose T_2 :Dextrose T_3 : Sucrose T_4: L-asparagine
T_5 : Glycine T_6 : Starch T_7 : Xylose T_8 : Maltose T_9 : Control

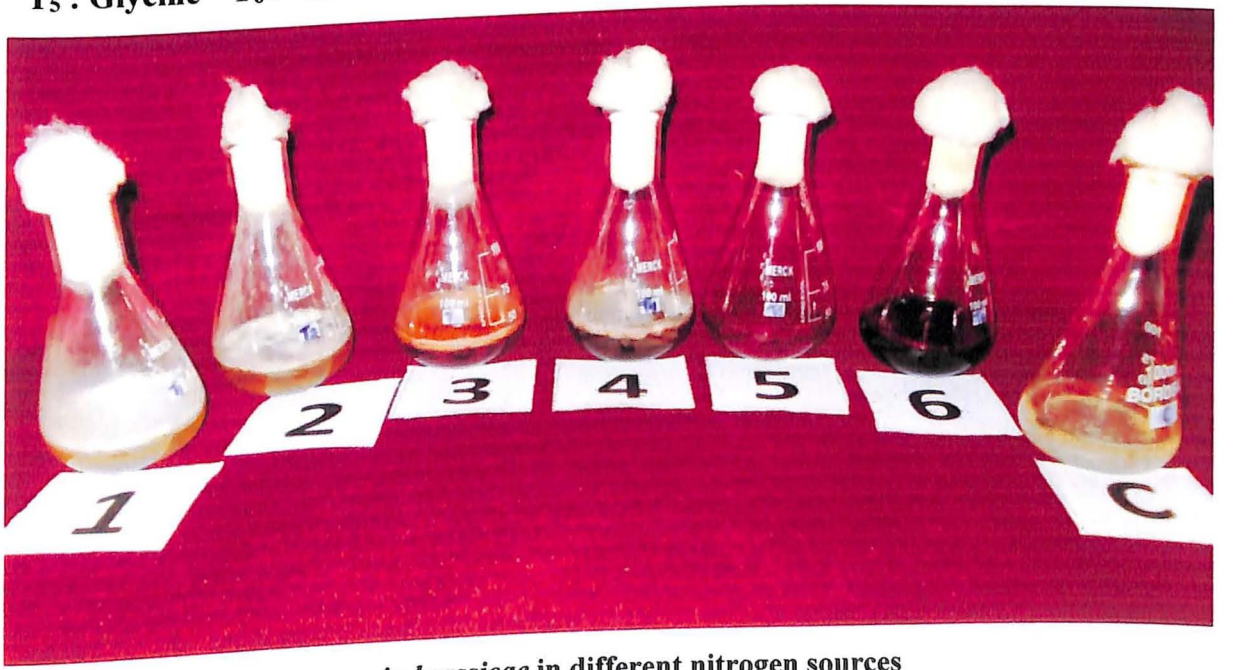

Fig. 16 Growth of *Alternaria brassicae* in different nitrogen sources

T_1 : Potassium nitrate T_2 : Sodium nitrate T_3 : Ammonium oxalate
T_4 : L-asparagine T_5 : Ammonium carbonate T_6 : Ammonium persulphate, C : Control

4.6 Physiological Studies

4.6.1 Effect of hydrogen ion concentration on the growth of *Alternaria brassicae*

Hydrogen ion concentration plays a significant role in promoting the vegetative growth as well as sporulation in fungi. In order to ascertain the optimum pH requirement of *Alternaria brassicae*, seven different pH regimes were evaluated *in vitro* in terms of the mean dry mycelial weight of test fungus at ten days of inoculation in Richard's medium. The data indicated that pH 7.0 was significantly superior in yielding the maximum mean dry mycelial weight growth of 360mg. Further, It was observed that both the highly acidic(pH 3.0 and 4.0) and the alkaline (pH 9.0) ranges didn't perform well in promoting the vegetative growth of the test fungus with the mean dry mycelial weight growth of 123.40, 153.42 and 146.21mg respectively. The causal fungus was observed to grow well in the pH range of 5.0-8.0 indicating that it could grow well in neutral to alkaline pH (Table 7 and Figure 17).

Table 7 Effect of different levels of pH on the growth of *Alternaria brassicae*

Sl. No.	pH of the medium	Mean dry mycelia weight(mg)
1	3.0	123.40
2	4.0	153.42
3	5.0	216.97
4	6.0	226.67
5	7.0	360.00
6	8.0	210.02
7	9.0	146.21
SEm±		9.317
CD (5 %)		28.26
CV%		7.86

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6	8.0	210.02
7	9.0	146.21
SEm±		9.317
CD (5 %)		28.26
CV%		7.86

4.6.2 Effect of photo period on the growth of *Alternaria brassicae*

Besides temperature and atmospheric humidity, photoperiod also plays a significant role in the build up of disease epiphytotic. In order to find out the appropriate photoperiod required for the growth expression of the fungus, five photoperiods including absolute light and darkness were evaluated *in vitro* in terms of the linear mycelial growth in pertriplates. The data revealed that 16 hours of light along with 8 hours of darkness could facilitate maximum mean diameter growth (69.97mm) of the test fungus followed by 12 hours of light along with 12 hours dark (68.92mm), both being statistically at par with each other. Indicating the fact that, 12-16 hours of photoperiod is necessary for optimum growth of the majority of disease causing fungi. Further, the least diameter growth of 54.89mm was supported by the condition of 24 hours dark which was statistically at par with that subjected to 8 hours light and 16 hours dark with 58.00mm radial growth of the test fungus. The finding of the investigation revealed that photoperiod as a single function didn't influence much on the mycelial growth of the test fungus. (Table 8 and Figure 18).

Table 8 Effect of light on the growth of *Alternaria brassicae*

Sl. No.	Treatments(Duration of time)	Mean radial growth (mm)
1	24h light	62.45
2	12h light 12h dark	68.92
3	16h light 8h dark	69.97
4	8h light 16h dark	58.00
5	24h dark	54.89
SEm±		2.286
CD (5%)		6.954
CV%		7.275

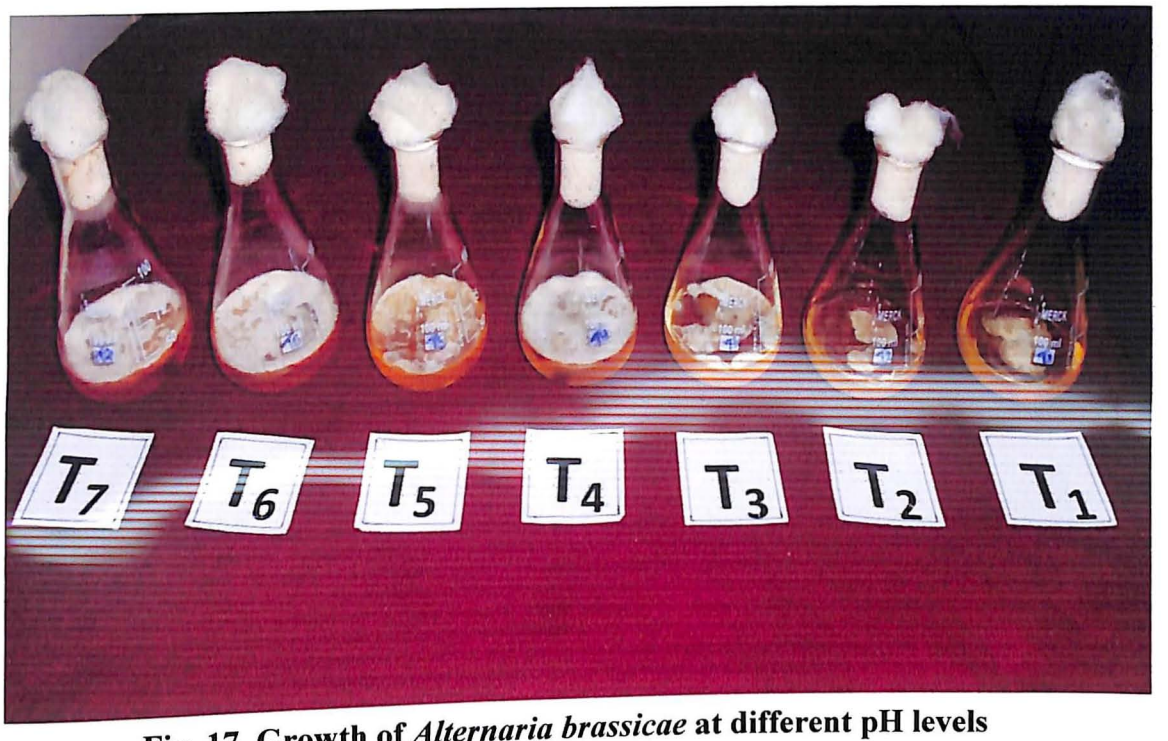


Fig. 17 Growth of *Alternaria brassicae* at different pH levels

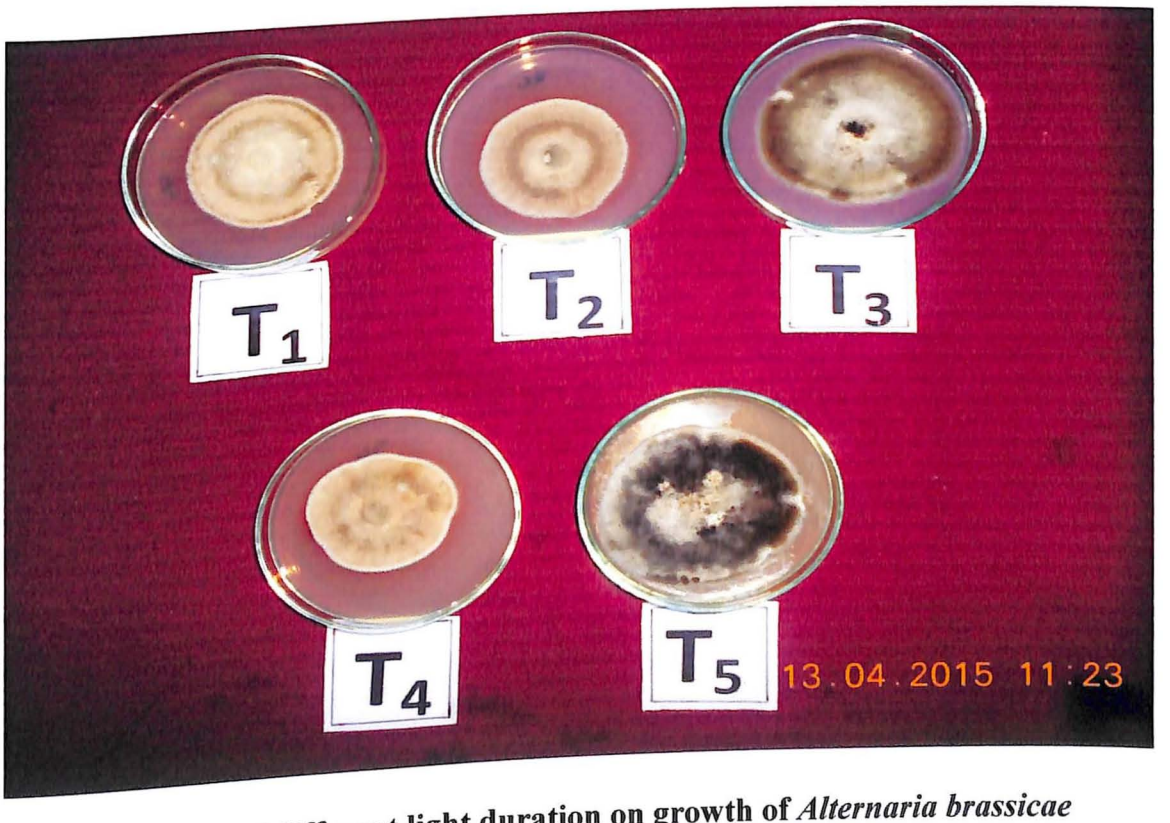


Fig. 18 Effect of different light duration on growth of *Alternaria brassicae*

4.7 Disease management studies

4.7.1 *In vitro* evaluation of phytoextracts against *Alternaria brassicae*

In order to find out an eco-friendly disease management schedule against *Alternaria brassicae*, 11 different phytoextracts in two concentrations (10% and 20%) along with control were evaluated *in vitro* adopting poisoned food technique.

Data in the table revealed that, all the plant extracts inhibited mycelial growth of the fungus at 10% and 20% concentrations and were significantly superior over control. The results indicated that, the maximum inhibition was recorded by Eucalyptus (67.73-76.81%) at both 10% and 20% concentrations over control followed by Datura (63.71-70.60%) and Deodar (49.91-58.05%). The least reduction of growth was observed in Calotropis (35.12%) and Bisalyakarani (33.02%) at 10% and 20% concentration respectively. Hence, the above laboratory experiment suggested that Eucalyptus, Datura, and Deodar could possibly be used in the management of *Alternaria* blight of cabbage caused by *Alternaria brassicae* (Table 9, Figure 19).

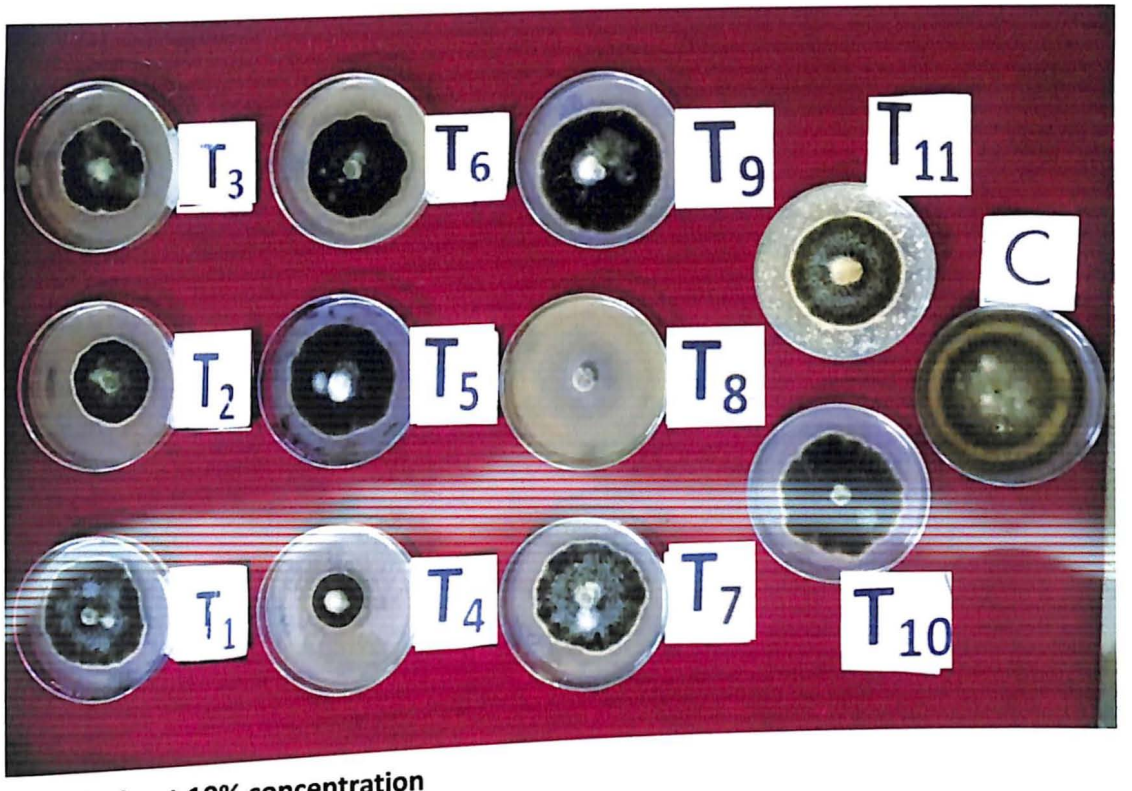
4.7.2. *In vitro* evaluation of oil against *Alternaria brassicae*

The plant essential oils are the potential sources of the antimicrobials of natural origin that have recently gained a considerable importance in management of important plant diseases. In view of this, a trial was designed to evaluate six essential oils for their performance in inhibiting the mycelial growth of the test fungus *Alternaria brassicae*. Analysis of data indicated that, Clove oil at all the three concentrations (0.5, 0.75, and 1.0%) was significantly superior in inhibiting growth of the test fungus to the tune of 100%. This was followed by Karanj oil with the percentage inhibition of 38.63-74.84%. However the essential oils out of Neem, Eucalyptus, Castor and Olive oil were not that effective could in inhibiting the mycelial growth. Hence, the finding of this investigation could be useful in promoting the essential oils as potential antimicrobial agents, preferably the Clove (Table 10 Figure 20).

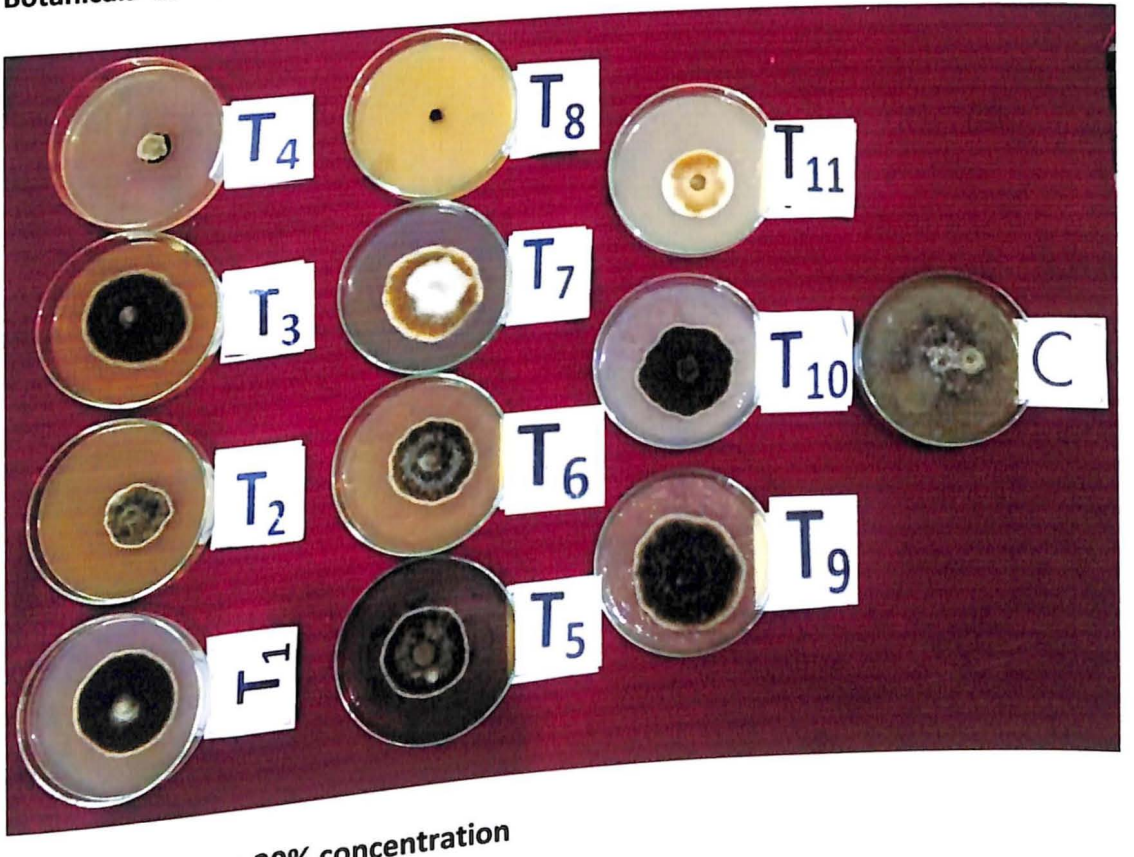
Table 9 *In vitro* evaluation of different phytoextracts against *Alternaria brassicae*

Sl. No.	Botanicals	Radial growth of the fungus(mm)		Mean Per cent inhibition of mycelial growth	
		10%	20%	10%	20%
1	Milkweed *Calotropis procera*	69.67	57.56	21.48 (27.56)	35.12 (36.33)**
2	Deodar *Polyalthia longifolia*	44.44	37.22	49.91 (44.94)	58.05 (49.60)
3	Karanj *Pongamia pinnata*	50.5	45.7	43.08 (40.98)	48.49 (44.08)
4	Datura *Datura stramonium*	32.2	26.08	63.71 (52.95)	70.60 (57.17)
5	Lantana *Lantana camara*	63.9	51.78	27.98 (31.88)	41.64 (40.16)
6	Neem *Azadirachta indica*	54.67	47.22	38.38 (38.23)	46.78 (43.11)
7	Onion *Allium cepa*	62.9	57	29.11 (32.65)	35.76 (36.69)
8	Eucalyptus *Eucalyptus globules*	28.67	20.57	67.73 (55.37)	76.81 (61.21)
9	Bisalyakarani *Tridex procumbens*	63.2	59.43	28.77 (32.39)	33.02 (35.06)
10	Tulsi *Ocimum sanctum*	62.77	58.3	29.25 (32.71)	34.29 (35.79)
11	Garlic *Allium sativum*	55.8	44.87	37.11 (37.52)	49.43 (44.66)
12	Control	88.73	88.73	-	-
	SEm±	3.111	1.100	1.912	0.713
	CD (5%)	9.08	3.21	5.60	2.09
	CV%	9.54	3.85	7.04	2.90

** The figure with in parenthesis is angular transformed value



Botanicals at 10% concentration



Botanicals at 20% concentration

Fig. 19 Efficacy of plant extracts on growth of *Alternaria brassicae*

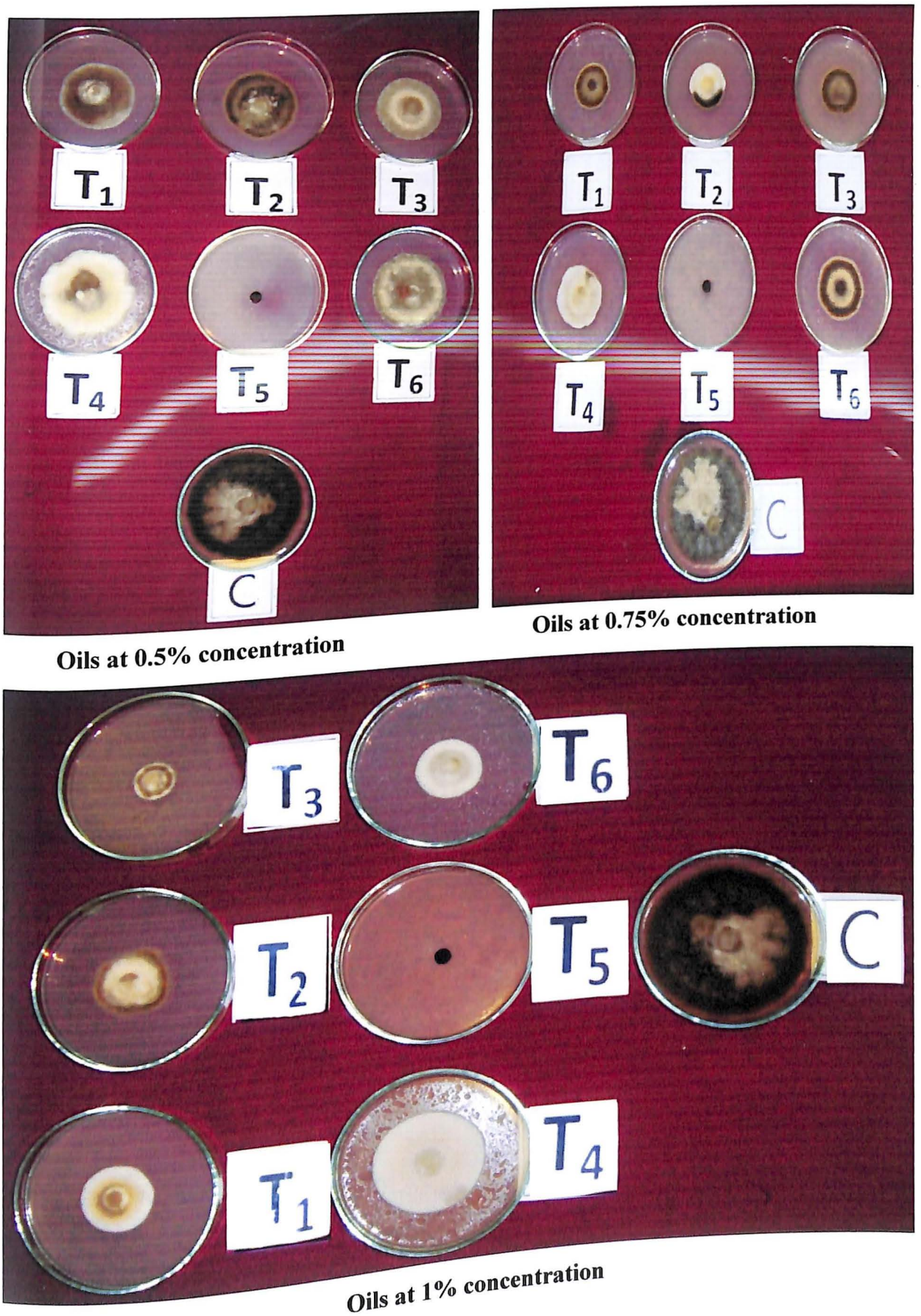


Fig. 20 Efficacy of different oils on the growth of *Alternaria brassicae*

4.7.3 *In vitro* evaluation of biocontrol agents against *Alternaria brassicae*

Biological control of plant pathogens with potential bioagents has been an important tool of non-chemical means of plant disease management. An exploratory study was under taken through *in vitro* screening of five bioagents which was conducted to ascertain their fungitoxic potential as suitable bio-pesticides against *Alternaria brassicae*. Analysis of data indicated that *Trichoderma viride* was superior in inhibiting growth of test fungus to the tune of 79.26% which was followed by *Trichoderma harzianum* recorded the mean percentage inhibition of 76.91%. The bacterial bioagents like *Pseudomonas fluorescens* and *Bacillus subtilis* were however, not found as potential bioagents in suppressing the growth of *Alternaria brassicae*. Hence the investigation revealed that, *Trichoderma viride* and *Trichoderma harzianum* could be exploited for their biocontrol potential against *Alternaria brassicae*. (Table 11 Figure 21).

Table 11 ***In vitro* evaluation of different biocontrol agents against *Alternaria brassicae***

Sl. No.	Bioagents	Mean radial growth of fungi(mm)	Per cent growth inhibition
T1	*Trichoderma viride*	18.66	79.26
T2	*Trichoderma harzianum*	20.78	76.91
T3	*Pseudomonas fluorescens*	23.67	73.7
T4	*Bacillus subtilis*	32.33	64.07
T5	*Beauveria bassiana*	36.33	59.63
T6	Control	90.00	-
	SEm±	1.252	
	CD (5%)	3.857	
	CV%	5.866	

4.7.4 *In vitro* evaluation of agrochemicals against *Alternaria brassicae*

In view of the fact that, chemicals are the last resort in the Integrated Disease Management schedule, A set of nine agrochemicals constituting of contact, systemic and the combinations there of along with control were evaluated *in vitro* against *Alternaria brassicae* adopting poisoned food technique. The results indicated a positive co-relation between the agrochemicals tested and the percent growth inhibition of test fungus. However, the test chemicals could inhibit the growth of the test fungus to varying extent (39.01-100%). Significantly maximum percent growth inhibition (100%) was observed in case of Propiconazole at 0.15% followed by Difenconazole (0.05%) and Fenamidione + Mancozeb (0.1%) associated with the percent growth inhibition of 84.16% and 83.75% respectively. It was further observed that chemicals like Azoxystrobin (0.1%), Chlorothalonil (0.2%), Copper hydroxide (0.1%), were inferior in inhibiting the mycelial of *Alternaria brassicae* in laboratory conditions. The overall growth inhibition of the test fungus in the investigation was recorded in the range of 39.01-100%. (Table 12, Figure 22).

Table 12 *In vitro* evaluation of different fungicides against *Alternaria brassicae*

Sl. No.	Trade name	Chemical name	Recommended dose (%)	Radial growth of fungus (mm)	% of growth inhibition
1	Saaf	Carbendazim12%WP+Mancozeb63%WP	0.2	27.26	69.71
2	Sectin	Fenamidione10%WP+Mancozeb	0.1	14.62	83.75
3	Kavach	Chlorothalonil 75%WP	0.2	46.33	48.52
4	Score	Difenconazole 25%EC	0.05	14.25	84.16
5	Kocide	Copper hydroxide 77%WP	0.1	38.56	57.15
6	Roko	Thiophanate Methyl 70% WP	0.15	31.41	65.1
7	Amistar	Azoxystrobin 23% EC	0.1	54.89	39.01
8	Nagcoper	Copper oxychloride	0.3	30.67	65.92
9	Tilt	Propiconazole 25% EC	0.15	0	100
10	Control			90	-
		SEm±		2.294	
		CD (5%)		6.78	
		CV%		11.20	

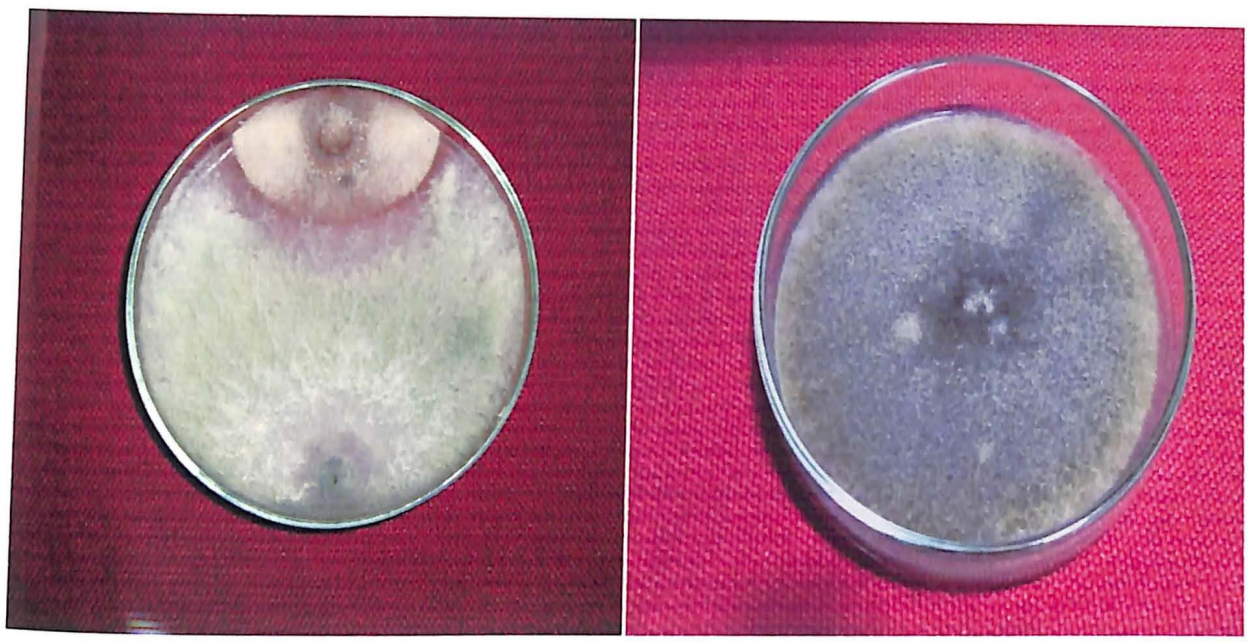


Fig. 21 Efficacay of *Trichoderma viride* against *Alternaria brassicae*

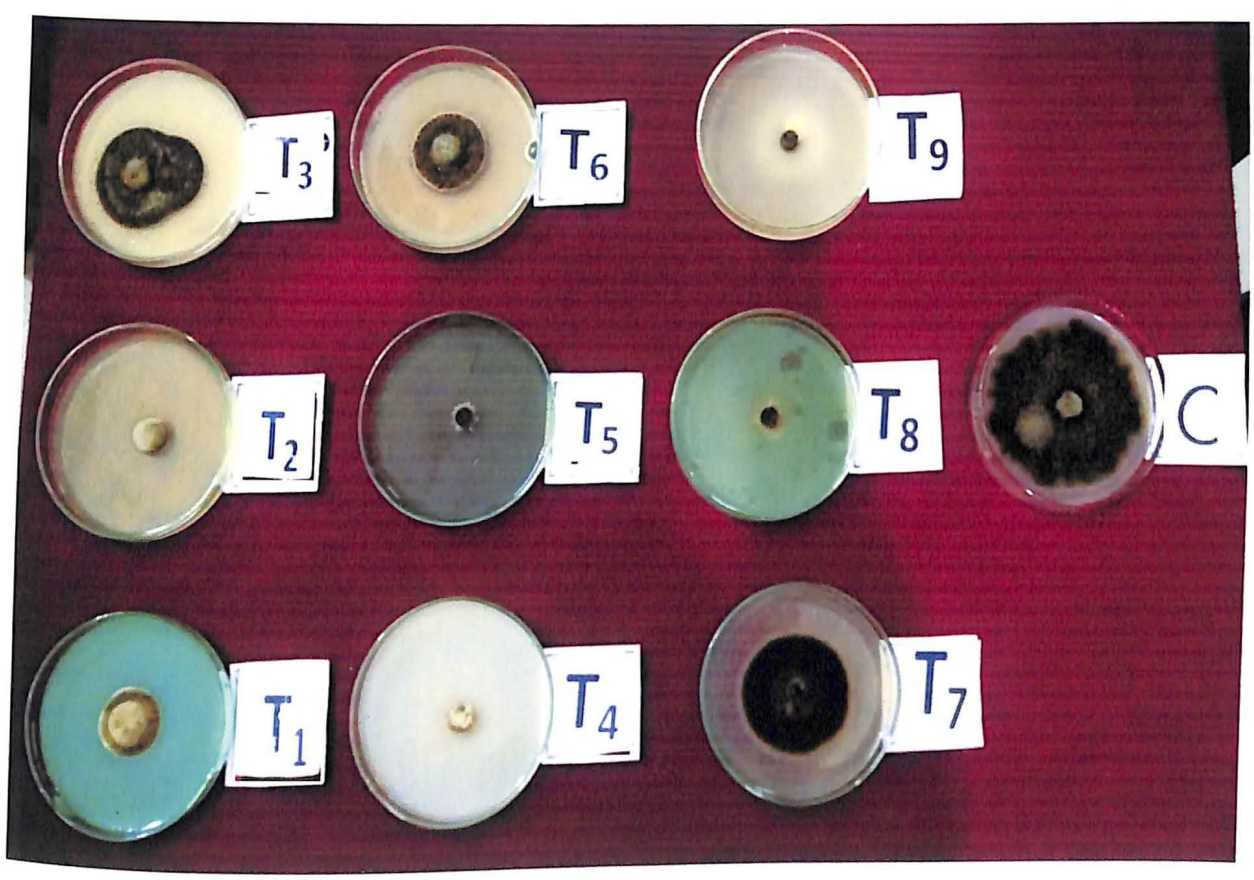


Fig. 22 Efficacy of different fungicides on growth of *Alternaria brassicae* under *in-vitro* condition

CHAPTER-V

DISCUSSION

DISCUSSION

In recent years, India is emerging as an important producer of vegetables and fruit crops. Among the vegetables, Cabbage is gradually gaining importance as it is grown almost throughout the year for its economic and nutritional value. The importance of outbreak of different diseases on this crop is major constraints for its low productivity. Cabbage is affected by various fungal as well as bacterial diseases like damping off, club root, downy mildew, *Sclerotinia* rot, black leg, black rot, soft rot and *Alternaria* blight. Under favourable climatic condition, diseases occur in alarming proportions leading to deterioration of yield and quality of heads. The disease *Alternaria* leaf blight of cabbage is prevalent in all the cabbage growing states and is one of the major problem. *Alternaria* blight caused by *Alternaria brassicae* was observed in and around Bhubaneswar was studied with various aspects like isolation, identification, proving pathogenicity of pathogen, cultural and morphological characteristics and management practices including phytoextracts, bio-agents and chemicals in *in vitro* conditions.

The infected fresh leaf samples were examined under microscope and the fungal pathogen isolated was identified as *Alternaria brassicae.* This has already been confirmed earlier by Babadoost and Gabrielson (1979), Cerkauskas *et al.* (1988) and Reis and Boiteux (2010), who reported *Alternaria brassicae was* the major leaf pathogen on brassicaceae.

The disease appeared at all stages of crop growth in cabbage. At nursery stage the seedlings develop spots on leaves resulting in damping off. The disease spreads to aerial parts of plant as minute yellow specks on leaves and stem, gradually darken and enlarge into circular, tan to black coloured concentric rings giving the appearance of target board surrounding by yellowish halo. In advance stage it changed to tan, brown or black in colour, papery in texture and finally falls off giving the appearance of shot hole. Kadian and Saharan (1984), Saharan and Mehta (2002) , Khan (2010) and Meena *et al.* (2010) reported the symptoms produced by *Alternaria brassicae* was very prominent, formation of concentric ring in the lesion with yellow halo zone. This finding is in agreement with our investigation.

The fungus associated in the diseased leaf was examined for its morphological features. The microscopic examination revealed the presence of brownish black, obclavate with 4-11 transverse and 0-4 longitudinal septate conidia. The conidia measured 28.85-67.28μm ×11.39-13.91 μm with an average of 41.90 ×12.94 μm including beak length. The conidia borne singly or in chains of 2-4. Conidiophores were found long distinctly geniculate, arise in fascicles, dark brown, unbranched, erect and 1-5 septate. Such findings are also in the agreements with the findings of Kolte (1985) and Kiprop *et al.* (2009) who reported the morphological characters of *Alternaria brassicae* in *Brassicae spp.* Ramjegathesh and Ebenezar (2012) and Sharma *et al.* (2013) earlier described similar characteristics of *Alternaria alternata* and *Alternaria brassicae* infecting onion and mustard respectively.

On pathogenicity test, the causal fungus could induce typical symptom on test plant after a week of artificial inoculation where as the plant without inoculation did not exhibit any symptom. Scholze (2004) has confirmed pathogenicity of *Alternaria brassicae* on four cabbage cultivars. Akhtar et al. (2004), Mangala et al. (2006) and Tziros et al. (2008) has proved pathogenicity of *Alternaria alternata* on tomato, chilli and pomegranate, respectively. Thus the pathogenicity of *A. brassicae* on cabbage has been proved in the present study which confirmed the above earlier reports.

In order to obtain adequate knowledge on the pathogen concerning growth characteristics , it was grown on a variety of semisolid and liquid media, the data presented earlier have shown that the causal organisms (*Alternaria brassicae)* exhibited most superior growth in Potato dextrose agar medium (85.89 mm) followed by Sabouraud dextrose agar medium (71.63mm). Where as least growth was recorded in Asthana and Hawker's agar (54.48mm). Sharma *et al.* (2013) investigated the growth requirement of *Alternaria brassicae* on different media and reported that the Potato dextrose agar supported the maximum mean radial growth of the fungus (>80mm) on the seventh day after inoculation. Maheswari *et al.* (2001), Singh *et al.* (2001), Akhtar *et al.* (2004), Pandey *et al.* (2006) and Isra Ram *et al.* (2007) revealed that maximum mycelial growth was obtained on Potato dextrose agar by *Alternaria alternata.* The least efficacy of Asthana and Hawker's medium has also been reported by Kumar and Singh (2003), which is corroborating our finding. With respect to

sporulation, the same trend was recorded as radial growth. It was also revealed that Richard's broth medium supported maximum dry weight growth (653.41mg) followed by Oat meal broth (640mg). Shekarappa (1999) reported the maximum growth on Richard's broth. However, Somappa *et al.* (2013) studied on *Alternaria solani,* growth and sporulation was maximum on Potato dextrose broth (34.1mg and 13.2×10^6 spores/ml) which contradict to our finding.

Evaluation of various carbon sources on the growth of *Alternaria brassicae* revealed, sucrose is the best source of dry mycelial weight growth (492.37mg) followed by maltose(423.48mg) and starch (413.33mg) in respect of mean dry mycelial weight growth (mg) of test fungus. However, carbon sources like glycine was not efficient growth promoters as recorded in the investigation. Mathur and Sarboy (1977) and Singh (2000) also reported sucrose as the carbon source that supported excellent vegetative growth, maximum sporulation in case of *Alternaria alternata* and *Alternaria porri.* Goel (1977), Maganhotto and Melo (1999) and Ramjegathesh *et al.* (2012) who reported that maltose supported the maximum dry weight growth of fungus (*Alternaria alternata* and *Alternaria solani*), which is in agreement with our investigation.

Among nitrogen sources tested for the growth of *Alternaria brassicae*, L-asparagine supported maximum dry mycelial weight (676.67mg). However, this was found statistically at par with three other nitrogen sources viz. ammonium oxalate, sodium nitrate and potassium nitrate. Hossain and Mian (2003) demonstrated the excellent performance of L-asparagine on the growth of *Alternaria brassicicola* causing leaf blight of cabbage which supports our finding.

The favorable reaction (pH) of the nutrient medium for the growth of the test fungus was studied by growing the fungus in different pH regimes from 3.0 to 9.0. The data revealed that, pH 6.0-7.0 was ideal for growth of the fungus. The pH 7.0 was best for the growth of the fungus (360.00mg). The earlier workers such Hasija (1970), Zhu *et al.* (1996), Mishra and Mishra (2012) and Devi *et al.* (2014) reported the ideal growth of fungus between pH 6.0 and 7.0.which corroborating our finding. Therefore it is suggested that high alkaline and high acidic behavior did not favour the growth of the pathogen. From photoperiod experiment it was found that 16 hours of light along with 8 hours of darkness facilitated maximum mycelial growth (69.97mm) followed by 12 hours of light along with 12 hours of darkness (68.97mm) indicating that 12-16

hours of photoperiod is necessary for optimum growth of the fungus. Gupta *et al.* (1972), Ansari *et al.*(1989), Prasad and Naik (2002) and Changkun *et al.* (2004) reported the similar finding, where as Singh *et al.* (2001) reported maximum conidial germination and sporulation in total darkness which contradicts our finding.

Among the non chemical means of crop disease management, use of phytoextract is important in view of its eco-friendly nature. Eleven plant extracts of different plant species were tested against *Alternaria brassicae* employing poison food technique at 10% and 20% concentration. Eucalyptus at both 10 and 20% concentration proved efficacious (67.73-76.81%) followed by Datura (63.71-70.60%) and Deodar (49.91-58.05%). The least reduction of growth was observed in Calotropis (21.48%) and Bisalyakarani (33.02%) at 10 and 20% concentration respectively. Eucalyptus, a potential plant extract found efficacious against *Alternaria brassicae* has reported earlier by Patni *et al.* (2005), Sasode (2012) and Ganie *et al.* (2013). The efficacy of Datura has been demonstrated successfully by Shivpuri *et al.* (1997) and Singh *et al.* (2007) which are in agreement with the present findings.

Essential oils of Neem, Eucalyptus, Karanj, Castor, Clove and Olive oil were tested for their efficacy against the growth of *Alternaria brassicae* at three concentrations (0.5, 0.75 and 1.0%). Among the oil tested, clove oil acts at the concentration of 0.5, 0.75, 1.0% was significantly superior than others which recorded 100% growth inhibition of the test fungus. Use of oil against the growth of *Alternaria* spp. causing *Alternaria* leaf spot of cabbage has been reported earlier by Yadav *et al.* (2014).The neem oil possessing antifungal activity has been reported earlier by Babu *et al.* (2000) and Vadivel and Ebenzar (2006) which also supports our finding. The antifungal activity of clove oil against *Fusarium sp., Diaporthe sp., Phomopsis sp.,Helminthosporium sp.,Colletotrichum sp. and Thanatephorus sp.* proved effective (Jasenka *et al.*,2010). Our finding says clove oil is also found highly effective against *Alternaria brassicae*, the incitant of cabbage leaf blight.

In the present study the antifungal characteristics of the bioagents such as *Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens, Bacillus subtilis* and *Beauveria bassiana* were tested *in vitro* to study effectiveness against *A. brassicae* employing dual culture technique. Maximum growth inhibition was recorded in *Trichoderma viride* (79.26%) followed by *Trichoderma harzianum*

(76.91%) but least inhibition was recorded in *Beauveria bassiana* (59.63 %). *Bacillus subtilis* and *Pseudomonas fluorescens* also appreciably inhibited the growth of the pathogen. The effectiveness of *Trichoderma* spp. was confirmed earlier by various workers such as Yadav *et al.* (2014) reported efficacy of the *T. viride* and *P. fluorescens* against *A. brassicae* causing leaf spot of cabbage, which are confirmed in the present study. However the efficacy of these bio control agents against other species of *Alternaria* has earlier reported by many workers like Abdul *et al.* (2001), Atia and Esh (2005), Harlapur *et al.*(2006), Kumar *et al.* (2006), Vadilal and Ebenezer (2006) and Singh and Kerkhi (2010), which are confirmed in the present investigation. The efficacy of *Pseudomonas* and *Bacillus* as potential antagonists has been reported earlier by Basim and Katircioglo (1990) and Leifert *et al.* (1992) which supports our findings. The entomopathogenic fungus *Beauveria bassiana* had the least effect against *Alternaria*, which is evident from the present investigation.

In integrated disease management schedule, use of chemicals are the last resort. Nine new chemicals were tested under laboratory conditions to determine the radial growth of *A. brassicae* adopting poison food technique. The results revealed a positive correlation between the agrochemicals and the per cent growth inhibition of test fungus. Maximum growth inhibition (100%) was recorded in case of Propiconazole @ 0.15% followed by Difenconazole (84.16%) and Fenamidione + Mancozeb (83.75%). The chemicals like Azoxystrobin 0.1%, Chlorothalonil 0.2% and Copper hydroxide 0.1% were not so effective against *A. brassicae*. The overall growth inhibition of the test fungus was recorded 39.01-100% in the present investigation. Harlapur *et al.* (2006), Nath (2012) and Bhargav *et al.* (2014) has reported Propiconazole @ 0.1% effectively inhibited the growth of *Alternaria*, which is in confirmity with our findings. The next best chemical Difenconazole 0.05% found efficacious against *Alternaria*, which supports the findings of Zhou *et al.* (2006) and Harde *et al.* (2014). The efficacy of Chlorothalonil 0.2% and Azoxystrobin 0.1% against *Alternaria* was found to be less by Waghe *et al.* (2014), while working on *A. helianthi* in Sunflower. It also supports our findings that both the chemicals i.e. Chlorothalonil and Azoxystrobin found least effective against *A. brassicae* infecting cabbage as evident from present investigation. However, the experimental finding has to be tested in field condition to ascertain the efficacy of test fungicides before recommendation to the growers.

CHAPTER-VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Leaf blight disease of cabbage is an important disease of observed throughout the state of Odisha. Samples collected from Central farm, Odisha University of Agriculture and Technology, Bhubaneswar, were found at all stages of the crop growth. At nursery stages the seedling develop spots on leaves resulting in damping off. The disease spreads to aerial parts of plant as minute yellow specks on leaves and stem, gradually darken and enlarge into circular, tan to black coloured concentric rings giving the appearance of target board surrounding by yellowish halo. In advance stage it changed to tan, brown or black in colour, papery in texture and finally falls off giving the appearance of shot hole which reduced the yield and quality of the crop.

The pathogen was isolated from all the above described symptoms and frequent isolation yielded the same fungus which was isolated and identified as *Alternaria brassicae*. The pathogenicity test was carried out by using the isolated pathogen which developed symptoms those were almost similar to the original symptoms of the sample. Reisolation from the artificially inoculated diseased plants yielded the same fungus which was found to be identical to the originally isolated fungus.

Study on the growth of the fungus in different solid media revealed that Potato dextrose agar medium supported significantly the maximum radial growth (85.89mm) of the test fungus followed by Sabouraud dextrose agar medium (76.11mm) and Malt extract agar (71.63). The other nutrient media namely Richard's agar, Host extract agar, Oat meal agar, Carrot root extract agar, Czapek's agar, Asthana & Hawker's agar were found inferior in respect of radial growth of fungus with the mean colony diameter of 68.11, 65.28, 64.14, 58.4, 56.99 and 54.48mm respectively. Among different liquid media tested that Richard's broth medium supported maximum dry weight growth (653.41mg) of the test fungus followed by Oat meal broth (640mg) and were statistically superior over the other liquid media. Minimum dry mycelial weight was obtained in case of Asthana & Hawker's broth (96.67mg) followed by host extract broth (136.67mg).

Among the different carbon sources evaluated for promoting the growth of the test fungus, sucrose was found superior (492.37mg) followed by that of maltose

(423.48mg) & starch (413.33mg). Further, it was found that carbon sources like L-asparagine and glycine were inferior in supporting the vegetative growth of the test fungus yielding the corresponding mean dry weight of 186.67 mg & 140.12 mg respectively.

The investigation on the effect of nitrogen sources on the growth of *Alternaria brassicae* revealed, L-asparagine as the best source giving a mean dry mycelial growth of (676.67mg) followed by ammonium oxalate (600.12mg), sodium nitrate (516.67mg) and potassium nitrate (510.23mg). Further, ammonium carbonate, ammonium persulphate and Richard's medium without any nitrogen source (control) was found to be inferior in expressing the mycelial growth of test fungus with the mean dry mycelial weight of 243.33, 176.85 and 146.67mg respectively.

The growth of the test fungus was subjected to seven different pH regimes 3.0 to 9.0 for an experimental period of 10 days. It was observed that a pH range of 6.0 to 7.0 was favourable for the growth of the fungus. The pH 7.0 was best for the growth of the fungus (360.00mg). Therefore it is concluded that the high alkaline and high acidic behavior did not favour the growth of the pathogen.

While studying the influence of light on the growth of *A. brassicae*, the cultural plate exposed to 16 hours of light along with 8 hours of darkness facilitated maximum mycelial growth (69.97mm) followed by the plates subjected to 12 hours of light along with 12 hours of darkness (68.97mm). This indicating that 12-16 hours of photoperiod is necessary for optimum growth of the fungus.

Among the eleven botanicals tested for their efficacy against the test pathogen at two different concentration (10% and 20%), leaf extract of Eucalyptus at both the concentration inhibited the growth to the maximum extent (67.73-76.81%) followed by the leaf extract of Datura (63.71-70.60%) and Deodar (49.91-58.05%). The least inhibitory effect was recorded by the leaf extract of Calotropis (21.48-35.12%) and Bisalyakarani (28.77-33.02%) at the same concentrations.

Among the oils tested against *A. brassicae* cloveoil was found absolutely efficacious in inhibiting the test fungus (100%) at all the three concentrations (0.5, 0.75 and 1.0%).The other six treatments including control were found unsuitable in suppressing the mycelial growth of the test fungus.

In an exploratory investigation, five biocontrol agents including two bacterial origins were evaluated for their efficacy against growth inhibition of *A. brassicae*, employing dual culture technique. It was observed that the mean per cent inhibition of test fungus was in the range of 59.63 to 79.26% among the bioagents tested.

Study on the efficacy of nine agrochemicals on the radial growth inhibition of the test fungus employing poisoned food technique revealed Propiconazole @ 0.15% was found absolutely inhibitory (100%) to the test fungus followed by Difenconazole (0.05%) and Fenamidione + Mancozeb (0.1%), associated with the per cent growth inhibition of 84.16% and 83.75% respectively. It was further observed that chemicals like Azoxystrobin (0.1%), Chlorothalonil (0.2%) and Copper hydroxide (0.1%) were inferior in inhibiting the mycelial growth of *Alternaria brassicae*. This result gives an option to choose the least toxic chemicals on need based basis for management of the disease.

As the disease is becoming serious in the state, the information generated in the present study on the management strategies is not sufficient to develop a concrete management package to the farmers. Therefore, further research work is needed to be undertaken on the other aspects of disease cycle of the fungus and its management giving more emphasis on ecofriendly cultural and biological aspects in order to develop an integrated disease management strategy against *Alternaria* blight of cabbage.

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