EFFECT OF TEMPERATURE ON GROWTH AND SPORULATION OF *ALTERNARIA* SPECIES FROM MUSTARD (*BRASSICA* SPP.) LEAF

By

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DECEMBER, 2015

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CERTIFICATE

This is to certify that the thesis entitled, "**EFFECT OF TEMPERATURE ON GROWTH AND SPORULATION OF *ALTERNARIA* SPECIES FROM MUSTARD (*BRASSICA* SPP.) LEAF**" submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN PLANT PATHOLOGY ,embodies the results of a labratory research work carried out by Rufaida Monowara bearing Registration No. 14-06362 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged.

Dated: 1st December, 2016
Place: Dhaka, Bangladesh

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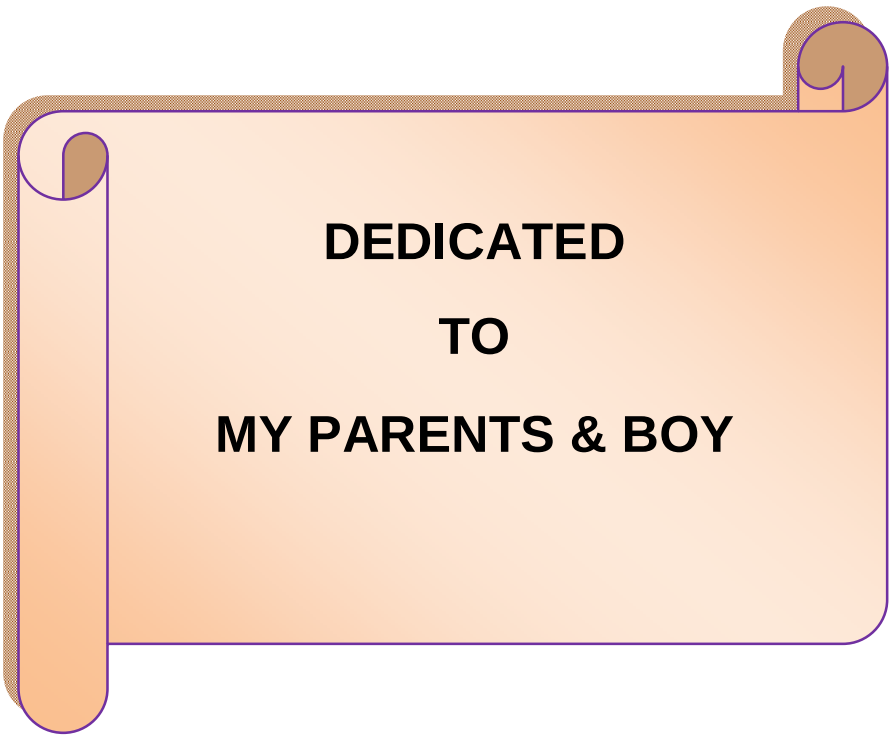
DEDICATED
TO
MY PARENTS & BOY

Acknowledgement

First of all I would like to thank almighty Allah, the most merciful and compassionate, the most gracious and beneficient to whom every praise is due and to his prophet Mohammad (SM).

Now I would like to pay ineffable gratitude to my supervisor Professor Dr. Nazneen Sultana, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. I am grateful to her ever inspiring guidance, keen interest, scholary comments, scholastic and constructive suggestions and polite behaviour throughout the course of my study.

I am especially grateful to my esteemed Co-supervisor Dr. Nazmoon Naher Tonu, Associate Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, for her proper guidance, inspiring cooperation and encouragement during the research work and preparation of thesis.

I am grateful and cordially express my thanks to my respected teachers Professor Mrs. Nasim Akhtar, Professor Dr. Md. Rafiqul Islam, Professor Dr. M. Salahuddin M. Chowdhury, Professor Dr. F. M. Aminuzzaman, Associate Professor Khadija Akhater, Associate Professor, Dr. Md. Belal Hossain, Associate Professor Dr. Fatema Begum, Associate Professor Abu Noman Faruq Ahmmed, Assistant Professor Sukti Rani Chowdhury, Assistant Professor Md. Ziaur Rahman Bhuiyan, Sayed Mohammad Mohsin, Lecturer, Hosna Ara Chowdhury Nisha, Lecturer, Department of Plant Pathology , Sher-e-Bangla Agricultural Unuversity, for their direct and indirect suggestin and encouragement and support during the whole exiperiment period.

I am thankful to Prof. Dr.Md. Anwarul Haque Beg, Dept. of Poultry Science, Sher-e-Bangla Agricultural Unuversity for giving me permission of using compound microscope.

I am greatful to Nazneen Ara Sultana, Senior Scientific Officer , Oilseed Research Centre, for her excellance cooperation during experiment and Ajhar Laboratory Assiatant, Oilseed Research Centre, for his cordial help.

I am satisfied to thank all stuffs of Plant pathology dept. and for their valuable and sincere help in carrying out the research work.

I also express my especial thanks to my friend Nishat, Ratan, Khalid, Nusrat and Shohan for their help and support during my work.

I found no words to thank my parents, sister, brothers, husband and my baby boy for their endless encouragement and continuous support, their sacrifice, untiring efforts to fulfill my dream of higher education. They were constant source of inspiration in the critical moments of my studies.

The Author

Effect of temperature on growth and sporulation of *Alternaria* species from Mustard (*Brassica* spp.) Leaf

By

Rufaida Monowara

Abstract

An experiment was conducted at Plant Pathology Laboratory, Oilseed Research center, Bangladesh Agricultural Research Inistitute (BARI), Joydevpur, Gazipur, Bangladesh to find out the effect of temperature on growth and sporulation of *Alternaria* species the causal agent of Alternaria leaf blight of mustard from 10 representative geographical locations of Bangladesh, during November 2015 to March 2016. All the isolates showed high level of variability *in vitro* in respect of radial mycial growth, colony colour, sub surface colour, colony shape, colony texture, zonation (surface and sub surface), length and width of conidia, beak length and number of septa. The maximum and minimum radial mycial growth was recorded 90 mm in isolate NAT_{Ab} and 83.67 mm in isolate GAZ_{Ab}, respectively at 14 days after incubation. Significant variation in conidial length, width, beak and no. of conidia observed in all isolates. The length of conidia ranged from 41.56 to 117.54µm with 3 to 11 transverse and 0 to 3 vertical septa. The width and beak length varied from 10.34 to 23.12 µm and 16.78 to 72.65 µm ,respectively. Surface colour were olivacious green to black and circular shaped colonies were observed in all isolates on PDA medium. Colony texture were cottony to velvety. Subsurface colour varied from light brown to black and pinkish. Zonation found in some isolates and some did not produce on both surface and subsurface. All conidia were murifrom and light brown to deep brown in colour. Potato Carrot Dextrose Agar mdium (PCDA) and 25 ^{o} C temperature were found optimum for different isolates for mycelial growth and sporulation.

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List of Symbols and Abbreviations

et al.	=	And others
spp.	=	Species
J.	=	Journal
no.	=	Number
etc	=	Etcetera
˚C	=	Degree Celsius
ml	=	Milliliter
µm	=	Micrometer
/	=	Per
SAU	=	Sher-e-Bangla Agricultural University
BAU	=	Bangladesh Agricultural University
BARI	=	Bangladesh Agricultural Research Institute
PCDA	=	Potato Carrot Dextrose Agar
CDA	=	Carrot Dextrose Agar
PDA	=	Potato Dextrose Agar
LSD	=	Least Significant Differences
CV%	=	Co-efficient of Variance
DAI	=	Days After Incubation

INTRODUCTION

Mustard (*Brassica* spp.) is the principal oil-producing crop of Bangladesh yielding 77.51% (BBS, 2015) of total oilseed production from 60.3% of the total area coverage. This crop is cultivated, at present, in about 802882 acres. The production is about 359452 lac metric tons oil (BBS, 2015). The average yield of mustard is 447 Kg/ha (BBS, 2015). Total production and per hectare seed yield of this crop may be increased by using high yielding variety (HYV) and improved production technologies. The different varieties of mustard seed contain 40-44% oil and mustard oil cake contains 40% protein (Chowdhury and Hassan, 2013). Oil cake is a nutritious food items for cattle and fish. It is also a good organic fertilizer for crops. Dry mustard plants may be used as fuel.

Mustard is cultivated almost all over the world. It is grown in tropical as well as temperate agroclimatic zones and is best adapted to areas having a relatively cool, moist climate during the growing season. (Kumar *et al.*, 2014). Pests and disease are the major constrains in the production of crucifers. Among the disease, Alternaria leaf blight caused by *Alternaria* species is one of the major diseases of mustard (Meena *et al*., 2016, Selvamani *et al*., 2014, Jha *et al.*, 2013, Aneja *et al*., 2013, Fakir. 2008, Verma *et al.*, 1994, Degenhardt *et al.*, 1974). This disease reduces mustard yield upto 47% in India (Sharma, 2009). It is an economically important pathogen in western Canada (Bansal *et al.*, 1990), in several countries of Europe and southeast Asia. (Conn., 1990). It is a prominant disease in India, Australia, Canada, Africa, England, Germany, France, Sri Lanka, Spain and Sweden. (Ghasemi *et al*., 2013).

Spors are produced in chains or in branching fashions which are multicellular pigmented. The spores are broadest near the base and elongate beak taper gradually. Around the initial site of host leaf *Alternaria* morphologically produces

a series of concentric rings. (Anju *et al.,* 2013) The pathogen infects all aerial plant parts, reducing photosynthetic area and accelerating senescence and defoliation.

Alternaria species can affect plant species in all growth stages, including seed. At seedling stages the disease is characterized by dark stem lesions just after germination that leads damping – off, or stunted seedlings. The symptoms may vary with host and environment. Symptoms are appear on lower leaves with appearance of black point at first, at later the point enlarge to develop into prominent, round, concentric spots of various sizes. Later on siliqua and stem round black conspicuous spots appear. These spots may coalesce resulting complete blacking of silliqua or weakling of the stem with formation of elongated lesions. (Meena *et al.*, 2010).

Alternaria species is a necrotrophic pathogen produce lesion on leaves, stem and siliquae which affect seed quantity, quality by reducing oil content, seed size and seed colour (Duczek *et al.*, 1999). This disease may cause significant losses in both temperate and tropical Brassica crops. (Mathpal *et al.*, 2011) The pathogen remained viable in diseased plant debris and seeds of infected plants (Ansari., 1989).

The major aspects of biology of an organism is the morphological and physiological characters of an individual within a species. Although it is not frequent in asexually produced individuals of the progeny. Variability studies are important to document the changes occurring in populations and individuals as variability in morphological and physiological traits indicate the existence of different pathotypes (Meena *et al.*, 2014).

Anamorph form of this pathogen shows great variability in morphology, physiology and pathogenicity. Several researchers have reported existence of variability based on morphology, sporulation, growth and cultural characteristics. Although, studies on pathogenic variability are important for the development of pre-breeding populations (Meena *et al.,* 2010). However information are lacking regarding differences in aggressiveness/virulence in the pathogenic population isolated from different *Brassica* species.

Conservation of plant pathogen both *ex-situ* and *in-situ* are important. We know, every pathogen species has numerous biotypes, races or pathotypes with specific genes in the respective host plant (Thakur, 1999). Proper understanding in the variation of pathogen population is highly crucial in the process of breeding for resistance against a particular disease.

Objective:

To find out the effect of temperature on growth and sporulation of *Alternaria* species.

REVIEW OF LITERATURE

2.1 Symtomps and causal organism

Meena *et al.,* (2016) mentioned that Foliar diseases are one of the most important limiting factors for cultivation of oilseed Brassica in tropical and sub-tropical areas in India. *Alternaria brassicae* (Berk.) Sacc. causes heavy economic losses to oilseed *Brassica* (Latin name) in terms ofseed yield.

Meena *et al.,* (2015) said Alternaria Blight (AB) caused by *A. brassicae* and *A. brassicicola* is a major constraint in rapeseed-mustard production in India. To study the effect of nutrients and lower leaf removal in Indian mustard (*Brassica juncea* L.) on AB disease an experiment was conducted during 2009-10 and 2010-11. Results indicated that maximum AB reduction (26%) was observed by soil application of Potash at 40 kg haG1+Zinc sulphate at 25 kg haG1+Copper sulphate at 40 kg haG1+Sulphur at 10 kg haG1+Ridomil-MZ 72 as foliar spray at 0.25% was better when applied as foliar spray (21%).

Selvamani *et al.,* (2014) stated Black leaf spot caused by *A. brassicae* (Berk.) Sacc. is an important disease of crucifers. Under field conditions environmental factors influenced the progression of Alternaria leaf spot. There was periodical increase in lesion number and per cent disease index (PDI). Maximum and minimum temperature was positively correlated with disease development,but average temperature showed high degree of correlation than the minimum (1.5-15.3˚C) and maximum temperature (10-28˚C). The laboratory study indicated that optimum conditions for spore germination were (20-24˚C) with more than 90 percent relative humidity.

Anju (2013) stated that *Alternaria* morphologically produces a series of concentric rings at the initial site of attack of host plant. It has a destructive effect on Cucarbitaceae, Brassicaceae and Solanaceae. Due to Alternaria blight 32-57% average yield loss occure.

Aneja *et al.,* (2013) reported that in the world oilseed brassicas grown is second largest. The production of this crop is greatly hampered by the fungal diseases. *Alternaria* blight being one of the most devasating fungal disease. This pseudo-fungi, by causing foliar damage to the crop leads to yield reduction and severely deteriorates the oil quality.

Ghasemi *et al.,* (2013) mentioned that Alternaria blight disease severely damages to oil-producing species of *Brassica* spp. all over the world and reduces the quality and quantity of the oil.

Jha *et al.*, (2013) reported that Alternaria blight caused by *A. brassicae* is a major devastating disease of Indian mustard, causing significant reduction in seed yield. Maximum Alternaria blight severity on leaves and siliqua was observed during 12-18 February, 2013.

Nowocki (2012) stated that *Alternaria* pathogens usually cause black spot disease, leading to damping-off of seedlings, spotting of leaves of cabbages, blackleg of heads of cabbages, and spotting/browning of cauliflower curds and broccoli florets.

Meena *et al.* (2011) reported India, Indian mustard attacked by various diseases viz., Alternaria blight (*A. brassicae*), white rust (*Albugo candida*), powdery mildew (*Erysiphe cruciferarum*) and Sclerotinia rot (*Sclerotinia sclerotiorum*). It

is a challenged for plant pathologiests to identify the ecofriendly disease management.

Mathpal *et al.* (2011) *A. brassicae* is a necrotrophic pathogen produce lesion on leaves, stem and siliquae which affect seed quantity as well as quality by reducing oil content, seed size and seed colour. This disease may cause significant losses in both temperate and tropical Brassica crops.

Kohl *et al.* (2011) observed that *A. brassicicola* and *A. brassicae* (dark leaf spot) can infect leaves of Brussels sprouts resulting in yield losses. Infections of outer leaves of sprouts cause severe losses in quality. Crop residues can be a major primary inoculum source of the pathogens. *A. brassicae* population increased in stalks exposed on the soil surface. The observed variation in population sizes of the pathogens between individual pieces of crop residues indicates a stochastic spread of pathogens.

Meena (2010) reported that Alternaria blight disease caused by *A. brassicae* (Berk.) Sacc. has been reported from all the continents of the world affects most cruciferous crops and is one among the important diseases of rapeseed mustard causing severe yield losses. The pathogen is greatly influenced by weather, the highest disease incidence reported in wet seasons and in areas with relatively high rainfall. The disease are characterized by formation of spots on leaves, stem and siliqua. At seedlings dark stem lesions appear which results damping-off.

Khol *et al.* (2010) stated that Infections by *A. brassicicola* and *A. brassicae* can cause severe losses of yield and seed quality in organic seed production of *Brassica* vegetables. On pod tissues development of *A. brassicicola* and *A. brassicae* and developing seeds was followed and seed quality was assessed. Seed colonization by the pathogen increased slowly until maturation but sharply

increased during maturation. They were concluded that *A. brassicicola* and *A. brassicae* have the potential to infect pods and seeds soon after flowering. For the production of high quality seeds, producers must prevent such early infections. Therefore, new control measures are needed for use in organic cropping systems.

Chauhan (2009) observed that the best time for the diseased sample collection was January to April. Infected leaf samples were collected in cellophane bag. Samples were collected from the different sites of Srinagar Garhwal and brought to the laboratory for further studies.

Fakir (2008) stated that Alternaria leaf blight caused by *A. brassicae* and *A. brassicaecola* is a devastating seed brone disease of mustard. The pathogen generally infect initially the leaves. From the infected leaves it spreads to stem and siliqua.

Duczek *et al.* (1999) reported Alternaria black spot, caused by *A. brassicae* (Berk.) Sacc, is found worldwide and is endemic in the northern canola growing areas of the Canadian prairie provinces, where it cause major yield reductions of up to 36%. This disease also reduces seed quality by increasing the green seed count, reducing seed weight, and decreasing the percent germination in harvested seed.

Conn (1990) said the causal agent of Blackspot disease of rapeseed and mustard is *A. brassicae*. It is an economically important pathogen in western Canada, in several countries of Europe and Southeast Asia.

Bansal (1990) reported in western Canada Alternaria leaf spot caused by *A. brasscae* is an economically important disease. Thirty-five cultivars/strains belonging to six Brassica species were evaluated for their reaction to Alternaria

brassicae under laboratory conditions. Detached leaves were wounded and inoculated with a spore suspension, and incubated at room temperature for 4 days.

Humpherson *et al.* (1989) stated Humidities are required for sporulation in *A. brassicae* and *A. brassicicola* on naturally-infected leaf discs of oilseed rape and cabbage required humidities equal to or higher than 91.5% and 87% r.h. respectively. The optimum temperatures for sporulation were 18–24°C and 20–30°C for *A. brassicae* and *A. brassicicola* respecttively at which temperatures both fungi produced spores in 12–14 h. Above 24°C sporulation in *A. brassicae* was inhibited.

Humpherson (1989) stated *A. brassicae* and *A. brassicicola* lesions present on infected leaves of oilseed rape and cabbage placed outdoors on soil produced viable spores for as long as leaf tissues remained intact. For oilseed rape this was up to 8 wk and for cabbage up to 12 week.

Ansari (1989) examined the modes of survival and perpetuation of *A. brassicae* attacking rapeseed and mustard were examined. The pathogen remained viable in diseased plant debris and seeds of infected plants which served as primary sources of inoculum. The pathogen was internally seed-borne.

Humpherson (1985) tested samples of oilseed rape shown in the UK between 1981 and 1984 indicated that on average 25% of samples were infected with *A. brassicae*. Much infection by *Alternaria* spp. occurred on vegetable and forage brassica seed produced in the UK between 1979 and 1983. In *B. oleracea* types *A. brassicicola* occurred most frequently, affecting 88% of samples and up to 55% of seeds. *A. brassicae* was detected in 44% of *B. oleracea* samples and in up to 13% of seeds.

Humpherson (1983) surveyed of brassica seed crops in Essex and Suffolk showed that *Alternaria* spp. occurred in many crops of *Brassica oleracea* in the years 1977–1980 affecting up to 100% of pods in each year. *A. brassicicola* was the only species present in 1976 and was the domioant pathogen in succeeding years but *A. brassicae* increased in frequency from 1977, causing 24% of the pod infections on *B. oleracea* in 1980. The latter fungus was the dominant species in crops of oilseed rape (*B. napus*), the mean incidence of infected pods increasing from 0.5% in 1977 to 2.9% in 1980.

Maude *et al.* (1982) oserved in the spring *A. brassicicola* lesions present on overwintered leaf litter of *Brassica oleracea* seed production crops produced high concentrations of spores, these were able to initiate new infections on foliage and subsequently on inflorescences and pods. A vertical disease gradient developed in maturing crops, the lowest pods becoming infected first and infection spreading slowly upwards. Spores were produced abundantly after 20 h leaf wetness at a mean temperature of 13°C or more.

Degenhardt *et al.* (1982) conducted an experiment in Canada.They observed that *A. brassicicola* required high temperature for germination and infection than *A. brassicae* and *A.raphani.Alternaria* spp. require RH above 95% for conidial germination. With dew periods of 6-8h at temperatures below 15˚C *A. brassicicola* germinates very poorly

Maude *et al.* (1980) tested in Britain on samples of basic and commercial *Brassica oleracea* seed between 1976 and 1978 showed that many lots were infected with *A. brassicicola*. Seed harvested in 1976 and 1977 *A. brassicae* was uncommon in basic seed in these years and in commercial but was frequent in seed harvested in 1978. Most affected seeds were contaminated by surface-borne spores

and mycelium of *A. brassicicola* but many were internally infected by the fungus situated within the seed-coat and in some seeds in the embryo tissues.

Tewari and Skoropad (1979) were tested the antibiotics Polyoxin AL W.P. and Polyoxin Z W.P. as therapeutants and prophylactics in the blackspot disease of rapeseed caused by *A. brassicae* (Berk.) Sacc. The germination and viability of the conidia, and the growth of *A. brassicae* in agar strongly inhibited by both fungicides. Both in the field and under laboratory conditions. It is concluded that to control the blackspot disease of rapeseed polyoxins have the potential.

Degenhardt *et al.* (1974) tested two cultivars of rapeseed, Span (B'assica cumpestris, L.) and zephyr (8. ttapus, L.). The cultivars were inoculated at various intelvals, beginning at 36-days after seeding, with *A. brassicae, A. raphani*, or a combination of these fungal specie. Yield reductions in plants with a severe incidence of alternaria black spot.caused by different inoculation tleatments, were both species of *Alternaria*.70 to 42%: *A. brassicae* 63 to 42%. Reductions in kernel weight of severely diseased paints were significant. Significant reductions in oil content occurred. Protein content also reduced in all disease ranks of plants inoculated with *A . brassicae*.

2.2 Morphological and Cultural characters

Meena (2016) found Brassica leaf extract, alfa-alfa seed decoction, and potato carrot broth. Tomato agar and Brassica agar media were found suitable for the growth of *A. brassicae* isolates.

Saha (2016) collected 23 isolates of *A. brassicae* from different cultivars in Uttar Pradesh and characterized for cultural, morphological, pathogenic and molecular variations. The colony colour is white, dark brown to light brown and pinkish in

white. The maximum length of conidia ranged from 150-122 µm with 8-9 transverse and 2 vertical septation.

Shaharan *et al.* (2016) defined *A. brassicae* mycilium septate, brownish grey, conidia brownish black, obclavete, murifrom, produced singly or in chain 2-3. Length of conidia varied from 96 µm-114 µm, bredth varied from 17 µm-24 µm and beck length varied from 45 µm-65 µm. The transverse and longtidunal septation varied from 10-11 and 0-6 respectively.

Soo-Sang *et al.* (2016) found leaf spot symptoms on black chokeberry (*A. mali*) the initial symptoms on leave surfaces were brown small-circular spots with a yellow halo lesionand gradually the small spots were fused, all of infected leaves dropped eventually. A fungus were isolated from the initial lesion, and cultured on potato dextrose agar. Colony color on upper surface of plate varied from olive gray to charcoal gray. Size of conidia mostly extend to 19–50 µm × 5–9 µm in nature and 20–59 µm × 8–13 µm in culture, with 3–8 transverse septa and usually no longitudinal septa or only 1 longitudinal septa in 1–3 of the transverse compartments and also have a short or long beak.

Yadav *et al.* (2016) were identified the morphological variations on the basis of its morphological and cultural characters of *A.brassicae* isolates from four different locations. Colonies of all the isolates were moderately fast growing, amphigenous, ashy grey, fluffy, circular. Conidia were obclavate to muriform, ovate elongated singly on conidiophore, sometimes in short acropetal chain. Highest average conidial size (140×20.7 µm and 138.6×22.9 µm) with 8.2 and 6.8 average transverse septa and 2.0 and 1.2 average longitudinal septa respectively. Highest average beak length was recorded 54.2 µm. The maximum mean radial growth of fungus at temperatures of 25°C.

Mohsin *et al*. (2016) examined twenty seven (27) isolates of *Alternaria porri* for characterization of cultural, morphological and pathogenic variabilities, were isolated from diseased leaf samples collected from different onion growing regions of Bangladesh. *A. porri* colonies colony colour ranged between light to dark olivacious and grayish white with irregular, regular with concentric ring and regular without concentric ring shape. Margin of colonies were entire, irregular and wavy with effuse, fluffy and velvety texture. Morphological variation in conidia production was between 7.720×10^3 to 47.02×10^3 per mm^2 with sporulation time 3.33 to 11.00 days.

Singh *et al.* (2015) observed ten isolates of *A. brassicae* for studied morphological, physiological and cultural variation collected from different geographical region in India. All the isolates showed variability *in vitro* in respect of size and septation of conidiophores and conidia, beak length, effect of temperature on radial growth and liquid medium on mycelia fresh weight. The length and width of conidiophores varied from 36.8-36.4 µm and 4.73-6.58 µm respectively. Conidiophores septation varied from 4.53-6.25 in different isolates of *A. brassicae*. Conidial length and width varied from 104.02-142.47 µm, 11.62-216.95 µm respectively. Beak length varied from 43.35-70.57 µm, transverse and longitudinal septa varied from 6-8.3 µm and 0.25-2.75 µm respectively. Carrot Potato Agar was better for all the cultures.

Saharan *et al.* (2015) said the pathogenic variability in four species of *Alternaria* is reported to be governed by determinant attributes viz., pathological, symptomatological, morphological, cultural, nutritional, biochemical, genetical, molecular, proteome level, thermo, and fungicidal sensitivity. Three races of *A. brassicae* viz., RM-1, RM-2 and V-3 virulent on rapeseed-mustard group of crops were identified. While race RM-1 was avirulent only on *B. oleracea* var. *Capitata*, race RM-2 was avirulent on both *B. oleracea* var. *Capitata* and *B. oleracea* var.

Botrytis. Race V-3, from vegetable crops was most virulent on the all host differentials. Three *A. brassicae* isolates designated as A, C and D differed in their morphology, growth,sporulation, and cultural characteristics along with virulence on *B. carinata*. Four *A. brassicae* pathotypes from *B. juncea* were identified and designated as Bj-4, Bj-5, Bj-6 and Bj-7.

Ginoya *et al.* (2015) studied cultural and morphological variability on seven different media *viz.*, potato dextrose agar, host leaf extract agar, host fruit extract agar, oatmeal agar, Richards' agar, Czapek's Dox agar and Rose Bengal agar revealed considerable variation among the isolates of *A. alternata* indicated the existence of variability in the pathogen. Oatmeal agar and potato dextrose agar were found as an excellent media to support the growth and spore formation of isolates of *A. alternata*, respectively. Distinct differences in terms of conidial length, breadth, beak length and number of septa were recorded among eight isolates of *A. alternata*. The average conidial length varied from 16.93 to 59.24 µm and breadth ranges from 6.90 to 14.98 µm with beak length of 3.25 to 44.07 µm. The transverse septa varied from 2 to 10 and longitudinal septa varied from 0 to 4.

Nikam *et al.* (2015) examined eight isolates of *A. solani* for identify pathogenic, cultural, morphological and molecular variability. All isolates were pathogenic to tomato. Great variability were observed on cultural characterictis in respect of mycial growth, colony growth, colony colour, zonation and sporulation. The mycial growth varied from 65.80 mm - 88.50 mm. irregular smooth colonies with concentric zonation with brownish black and dark gryiesh, without zonation olivacious black and grey color observed. The average conidial size (L×B) were 42.18×15.18 µm and beck size were 13.10 µm. The maxium horizontal septa varied from 5-12 and minimum varied from 4-8. The vertical septa maximum varied from 1-4 and minimum 1-3.

Singh *et al.* (2015) stated study the cultural and pathogenic variations of *A. brassicae*, affected leaf samples were collected from ten different places for the isolation and purification. Ten isolates of *A. brassicae* grown on five different culture media viz. Potato dextrose agar, Oatmeal agar, Host leaf extract agar, Czapek - dox agar and Carrotjuice agar *A. brassicae* varied in their cultural behaviour ranging from fluffy to compressed, with wavy, smooth to rough margins. Colonies colour varied from black, brown, light brown to dark brown and growth varied from slow, medium to fast on different culture media. Variation in zonation and sporulation on different medium were also observed in the isolates. Among the media in general, the fastest growth of each fungal isolates were recorded on PDA (83.77 mm) as compare to others; while slowest growth was recorded on Czapek - dox agar (79.54 mm) medium.

Parmila (2014) observed 10 isolates of *A. brassicae* for morphological, cultural, pathogenic and molecular variation. Colour of colonies ranged between white, off white to light brown. Conidia length and width varied from 105-135 µm and 10 - 20 µm respectively. Horizontal septa varied from 6.9-9.4. Colony was circular in shape.

Aneja (2014) examined 55 isolates of *Alternaria* species collected from infected leaf samples. Among them 32, 20 and 3 isolates were *A. brassicae*, *A. brassicaecola* and *A. alternata* respectively. Septation space pattern 3-6, 2-6, 2-4, transverse and 0-2, 0-1, 2-3 longitudinal septation in *A. brassicae*, *A. brassicaecola* and *A. alternata* respectively. The spore size varies from 36.7-257.6 µm in *A. brassicae*. Mycelial growth varied between 30-80 mm in *A.brassicae*.

Kumar *et al*. (2014) found 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. Sporulation occurs between the temperatures of 8-24°C but optimum temperatures range between 16-24°C.

Sharma (2014) demonstrated detached leaves were properly washed under running tap water and then surface wiped off with 70% alcohol .

Chand (2014) found the conidia of *A. brassicicola* were muriform, olivaceous brown colour with nonexistent beaks. The size of conidia recorded 13-120 μm in length and 6-16μm in width. The shape of the conidia was cylindrical to obclavate with cross and longitudinal septa. Isolates showed light black and grey white colours in Host Extract Agar media. The temperature between 25-30^{o}C was found optimum for the growth and sporulation of *Alternaria*.

Tanya *et al.* (2014) made a studied that *A. solani* is an economically important pathogen causing diseases on Solanaceae crops. Significant morphological variations in length and breadth of conidia, numbers of horizontal, vertical and oblique septations were observed in the test isolates. The conidia varied from 20.68-43.10 x 10.53-17.99 μm in brinjal, 19.86-43.73x 7.52-13.05 μm in chilli, 21.5-33.21 x 8.03-17.85 μm in potato and 30.31-75.47 x 7.26-27.42 μm in tomato Five different media i.e., Czapek dox agar (CDA), Potato Carrot agar (PCA), Carrot Agar (CA), Potato Dextrose Agar (PDA) and oatmeal agar (OA) were prepared and used for the cultural studies of the fungus.

Swati *et al.* (2014) conducted an experiment in India to found the morphological, cultural, pathogenic and genetic variability in thirty two *A. brassicicola* (Schwein) Wiltshire isolates infecting cauliflower (*Brassicae oleracea* var *botrytis*) from different parts. Dark olivaceous black fungal colonies were observed with small, obpyrifom, septate, brown colored spores forming in chain having no beak. A

significant ($p<0.05$) morphological variability was found within the isolates in respect to conidia length, width and number of septa whereas less cultural variability was seen with respect to colony colour and growth.

Deep *et al.* (2014) observed thirty two isolates of *A brassicicola* for colony color and radial growth. Colony colour of *A. brassicicola* varied from olive green to dark olivacious black on PDA. Mycial color were brown. Obyifrom conidia having brown colour with smooth surface and short beck. Average conidial length varied from 32.57-40.08 µm. Average conidial breadth varied from 6.23-9.40 µm. Average horizontal varied from 1.5-3. No vertical septa observed.

Sharma (2013) found variation in morphology and cultural characteristics among 32 Indian geographical isolates of *A. brassicae*. All isolates showed variability *in vitro* in respect of conidial length, width and number of septa. Variation was found in mycial growth, sporulation in different nutrient media like Potato Dextrose Agar, Cauliflower Agar medium and Carrot Potato Agar good for all isolates..

Giri (2013) examined Conidia of *A. brassicae* were muriform, beaked, bottle shaped, and measuring 92.3-102.5 µm long and 10.5-20.5 µm wide. Conidia had 6-10 or even more transverse septa and a few or none longitudinal septa. The beak length varied from 41-51.25 µm long. *A. brassicae* was subcultured from the 7-day old culture on V-8 agar medium (10% V-8 juice, 0.02% CaCO3 and 2% agar) incu-bated at 22°C.

Kumari *et al*. (2013) demonastrate 4 isolates of *A. brassicae* (Berk.) Sacc., on the basis of conidial morphology, cultural characteristics, sporulation intensity and length of incubation period. The average length was 134.0-171.0 µm and breadth were19.0-26.1 µm. The horizontal septations ranged from 4 - 18 and vertical from 0 - 9.

Shakti *et al.* (2013) stated that pathogenic, morphological and cultural variability exist among 5 different isolates of *A.brassicae*, collected from five different places of Uttar Pradesh. The variation in radial growth sporulation and conidial septation revealed that the maximum radial growth (52.5 mm) and good sporulation were observed in isolates from Sarsaul and the minimum growth and fair sporulation was observed in isolates of Billhaur Kanpur district showing dark brown colony characteristics. The horizontal septation varied from 4-13 and vertical from 0-6. The septum distance between two septa and length of beak also showed some variation.

Sofi *et al.* (2013) stated Alternaria blotch, causal organism *A. mali*, causes severe foliar damage to apple trees in Kashmir. Twenty one (21) isolates of *A. mali* were collected from different locations. The pathogen was characterized for cultural, morphological, pathogenic and molecular variations. Colonies varied in their cultural behaviour ranging from velvety to cottony, mostly appressed, with regular to irregular margins. Colour of colonies ranged between light to dark olivacious. Isolates impregnated media with colour ranging between grey to brown. Growth rate of isolates was between 5.86 to 8.21 mm/day. Morphological variations in size, shape and septation of hyphae, conidiophore and conidia were observed in the isolates with significant variations in conidiophore and conidial septation. Average conidial size ranged from 21.36 to 31.74 x 8.34 to 14.48 µm.

Muthukumar and Venkatesh (2013) stated single-spore culturing of the fungus *A. alternata* on the basis of morphological characteristics. Colonies on PDA medium are fast growing, black toolivaceous-black or grayish colour. Mycelium is subhyaline, septate, branched and measure 3.3 to 5.2 µm size in diameter. Conidiophores are pale brown, fasciculate, simple or branched, straight or flexuous, septate, dark coloured, geniculate and 47 × 3.2 µm size. Conidia are

formed in chains of 2 to 4, muriform, short beaked, smooth walled, light brown in colour and 38 × 12 µm size.

Meena (2012) observed variation in morphological characteristics of different isolates in growth, shape, and pigmentation of colony, conidial measurement and number of septa. Conidial length, width and beak length varied from 106.7-285.9µm, 33.5-57.0 µm and 41.4-180.0 µm respectively. Number of horizontal septa varied from 3.2-8.0 and vertical 0.3-1.4.

Goyal *et al.* (2012) studied morphological and cultural variability among 13 isolates of *A. brassicae* provides information about morphological variation and favorable cultural conditions *in vitro*, which could result in severe infection of Alternaria blight due to isolates existing in different geographical regions of India. This also reflected the adaptation of the respective isolates to the ambient conditions in the different cropping areas, where the disease occurs in varied proportions in different years, which may have also induced the available cultural variability.

Remander *et al.* (2012) examined tweenty five isolates of *A. brassicae* based on different morphological characters like colony growth, spore size, spore septation and sporulation potential and grouped into 3 groups.One group had whitish brown,appressed fast growing colonies with high sporulation. Other group showed white or whitish with brown margin, slightly fluffy medium with sparse to low sporulation. Another group had gryies white, fluffy slow growing with moderate sporulation.

Ramjegathesh and Ebenezar (2012) demonstrated ten isolates of *A. alternata* causing leaf blight disease of onion were collected from ten different onion growing areas of Tamil Nadu. Morphological variation were found and the

conidia were muriform shape and light brown colour. The length of their conidia was varied from 30.99 to 42.47 µm. The width of the conidia was varied from 11.90 to 17.37 µm. All the isolates produced both beaked and unbeaked conidia. The beak length of conidia varied from 18.7 to 23.81 µm. The number of cells in each conidia varied from 2-9. All the isolates took 13-16 days for sporulation.

Singh *et al.* (2012) conducted an experiment in the year 2012-13 for identified cultural, morphological and pathogenic variability of different isolates of *A. solani*, causing Early Blight in Tomato. Radial growth was not significantly different for most of the isolates. Seven DAI (days after inoculation) highest radial growth has obtained 35.50 mm, Ten DAI maximum growth was observed 52.00 mmand Thirteen DAI maximum radial growth was same observed (88.75 mm). The maximum mean mycelial growth was observed in 57.83 mm followed by 57.66 mm and 56.83 mm. Isolates of *A. solani* depicted high variability in pigment production on PDA medium. Mycelial growth patterns were observed on PDA circular margin with smooth surfaced colony and some grew with irregular margin and rough surface.

Khalaf (2012) stated *A. solani* is known economically important and the casual agent of early blight on potato and tomato. Morphology and physiology characteristics of *A. solani* were investigated for identification and variability. The optimum pH levels of *A. solani* grow *in vitro* were 6-7 and the optimum growing temperatures of the isolates recovery in this study was 25 and 30°C. The mycelial width between 0.8-1.5 µm and the conidia are 35-75 µm in length and 10-20 µm in width and 2-7 transverse septa and 1-4 longitudinal septa. This study pointed that there was a variation in the population of *A. solani* isolated from Jordan valley based on morphology and physiology characteristics.

Goyal (2011) studied 13 representative Indian geographical isolates of 219 collections of *A. brassicae* for identified morphological and cultural variation. All the isolates showed high level of variability *in vitro* in respect of conidia length, width, beak length and number of septa. Temperatures ranges (25-30˚c; 15-35˚c) were found for mycial growth and sporulation respectively. Average conidial length varied from 31.2 - 51.8µm (range: 24.0–62.6 µm). Average conidial width varied from 6.7 - 9.6 µm (range: 4.8–12.0 µm). Average beak length from 8.2 - 20.6 µm (range: 4.8–33.6 µm). Number of transverse septa varied from 4.0 - 7.2 (range: 3–8). Number of longitudinal septa varied from 0 - 0.4 (range: 0–2).

Khan (2011) demonstrated morphological and physiological variations in isolates of *A. brassicae* and to evaluate the efficacy of fungicide (*in vitro*) on radial growth of the fungus and foliar sprays (field) against the disease severity on rapeseed-mustard. Morphological variations of *A. brassicae* obtained from various places were differed in their conidial size and septation. Physiological response of *A. brassicae* at varying temperatures, the fungus showed its maximum growth at 25°C followed 20°C and on different culture medium, maximum radial growth is on PDA. The fungus exhibited a descending trend in its growth at lower and higher regimes of temperature.

Jadav *et al.* (2011) found that in ten isolates of *A. macrospora* differ with each other in respect of morphological, pathogicaland molecular basis. The average mycelial width of isolates ranged from 3.0 to 3.40 µm. The size of conidia ranged from 20.81-56.23 x 9.2-27.10 µm with 1-6 transverse and 0-4 longitudinal septa.

Stean *et al.* (2011) conducted an experiment during 2008 and 2009 phytopathological isolations weredone from soybean plants and seed samples from several localities in Serbia. A total of 19 isolates of *Alternaria* spp. were isolated, 13 from the seed and 3 from both leaf and stem. In order to determine and

characterize isolates, cultural, morphological, molecular and pathogenic characteristics were thoroughly investigated. The slowest growth of the examined isolates was noted on Malt agar (MA) with average colony diameter of 42.9 mm after 7 days of incubation. On other two media (V8 and Potato Carrot Agar), colony growth was uniform and faster, with average diameter of 66.8 mm and 66.1 mm, respectively. Isolates of fungi form unbranched or poorly branched conidial (5-12 conidia in chain) chains on short unbranched conidiophores. Conidia are dark in colour, multicellular with 2-7 transverse and few longitudinal septae. They are of different size regarding the place of formation in the chain.

Khulbe *et al.* (2011) were studied the morphological and cultural diversity of twenty isolates of *A. brassicae* collected from different locations of *Tarai* region of Uttarakhand, infecting rapeseed and mustard. Results showed that there was a distinct difference among isolates in terms of mycelial growth, colony characters, conidial length and conidial beak length. The isolates showed varied growth from slow to fast with varying margin type and colony colour, The average conidial length varied from 21.00 µm to 298.00 µm. The average minimum conidial length was observed in 55.23 µm and maximum 152.17 µm. The beak length of conidia range varied from 12.00 µm to 144.00 µm .

Reis (2010) observed colonies were initially white, turning brown within 5 to 7 days from plating on media. Conidia were pale brown in colour and 8 to 16 transversal septa and 0 to 7 longitudinal septa. The beak length ranged from 26-162 µm, conidial length varied from 68 to 310 µm and width varied from 18-28 µm.

Daniel *et al.* (2008) demonestrated that conidia production is a problem in the study of *A. alternata* from citrus. They studied effect of light and culture media for conidia production of *A. alternata*. They obtained the most virulent conidia

produced on PDA medium at 28ºC under constant black fluorescent NUV lamp for 4 weeks.

Kumar *et al.* (2008) studied eleven isolates of *A. solani,* causal organism of early blight of tomato, and were collected from different agroclimatic conditions and these isolates were characterized for cultural, morphological, pathogenic and molecular variations. On potato dextrose agar medium the pigmentation varied from yellow, brown, black, brownish to greenish black in isolates of *A. solani.* Radial growth of all isolates ranged between 14.9 mm and 32.2 mm on PDA and 24.3 mm to 53.7 mm on three selective media i.e., ASM, V-8 juice agar and V-8 juice agar (synthetic) on the fourth day. The thickness of conidiogenous hyphae varied between 1.17 µm and 9.56 µm. Most of the isolates showed smooth mycelial growth with circular and irregular margin and without concentric zonation. Sporulation was not found in any of the isolates on four different nutrient media, whereas conidiogenous hyphal length was observed in V-8 juice agar medium only.

Kaur *et al.* (2007) studied 322 isolates of *A. brassicae,* which were made from alternaria black spot infested *Brassica* leaves collected from a wide geographic spread of north-west India. morphology, colony diameter and sporulation patterns. Variation was recorded among conidial length, breadth and beak length which range of 51.4-481.2 µm, 6.9-36.0 µm and 16.3 - 266.9 µm respectively. Average number of horizontal septa were 9.7, vertical septa were 0.8 and beak septa were 3.7 in conidia. Colony diameter 13.0-77.5 mm and sporulation were in range of and 1.0-4.0×10^6 spores / ml. Colonies were brown, white, and olevaceous green with smooth or wavy margins and thick velvety to sparse growth.

Verma *et al.* (2006) examined variability among isolates of *A. solani*, the causal agent of early blight of tomato, from Northern and Southern parts of India. They

observed conidial morphology, pathogenicity tests and random amplified polymorphic DNA (RAPD) techniques. The isolates varied with respect to size of conidia and number of septa. The average size of conidia varied from 150-224.9 µm x 12.4-17.2 µm. The number of horizontal (4-14), vertical (0-3) and beak (0-8) septa also varied among the isolates.

Yoo *et al.* (2005) stated the conidia of *A. brassicae* showing obclavate conidia 105 to 210 µm long and 20 to 30 µm thick, with 11 to 15 transverse septa and 0 to 3 longitudinal or oblique septa, predominantly with a pronounced beak 5 to 8 µm thick extending 0.3 to 0.5 µm of the length of the conidium.

Mehta (2003) reported that *Alternaria* blight is one of the yield limiting factors particularly in Northern India.he investigate different agro climatic zones of India to identify the pathogenic variability among *A. brassicae* isolates. He found that isolates differed from spore size and categorized into four groups i.e small (<100µm); medium (101-150 µm); long (151-200 µm) and very long (>200 µm). The breadth was observed in range of 13.5-36 µm. The number of horizontal septa varied between 5–13. The number of beak septa varied from 0-6.

Barry *et al.* (2002) examined 308 isolates of *Alternaria* spp. were collected from the five sample sites. Based on characteristics of single-spored colonies, all isolates were grouped into approximately four colony types. Group 1 consisted of colonies contain lettuce green to olive green and usually had a prominent (2 to 5 mm) white margin. Colony texture was felty to woolly. Group 2 isolates produced colonies whch were pale olive gray to olive gray, often with a very thin (1 to 2 mm) white margin. Colony texture was generally woolly to cottony. Group 3 isolates produced colonies visible with typically dark olive gray to iron gray to castor gray in color. The colony margin of these isolates was often wavy or torn. Colony texture was generally felty to woolly presentative culture. Group 4 isolates

produced colonies that were either white to pale gray or apricot orange in color. Colonies generally had a cottony texture. The undersurfaces of the colonies were usually orange or dark orange. No diffusible pigments or crystals were produced by these isolates. Isolates typically produced colonies over 70 mm in diameter after 7 to 10 days.

MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted in the Plant Pathology Laboratory, Oil Seed Research Center, Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur, Bangladesh.

3.2 Experimental Period

The experiment was conducted from November 2015 to March 2016.

3.3 Collection of leaf sample

Mustard leaves having typical symptoms (Fig-1) were collected from 10 mustard growing districts of Bangladesh namely Dhaka, Rajshahi, Natore, Naogaon, Bogra, Lalmonirhat, Gazipur. Rangpur, Pabna and Mymenshingh (Fig-3). The diseased leaves were cut from the plants grown in the field and put into a brown paper envelope. Then the brown paper envelopes of each collection were taken to the laboratory to isolate the causal organism.

Figure 1. Symptomps of Alternaria leaf blight of mustard

Figure 2. Collected Disease Leaf Sample

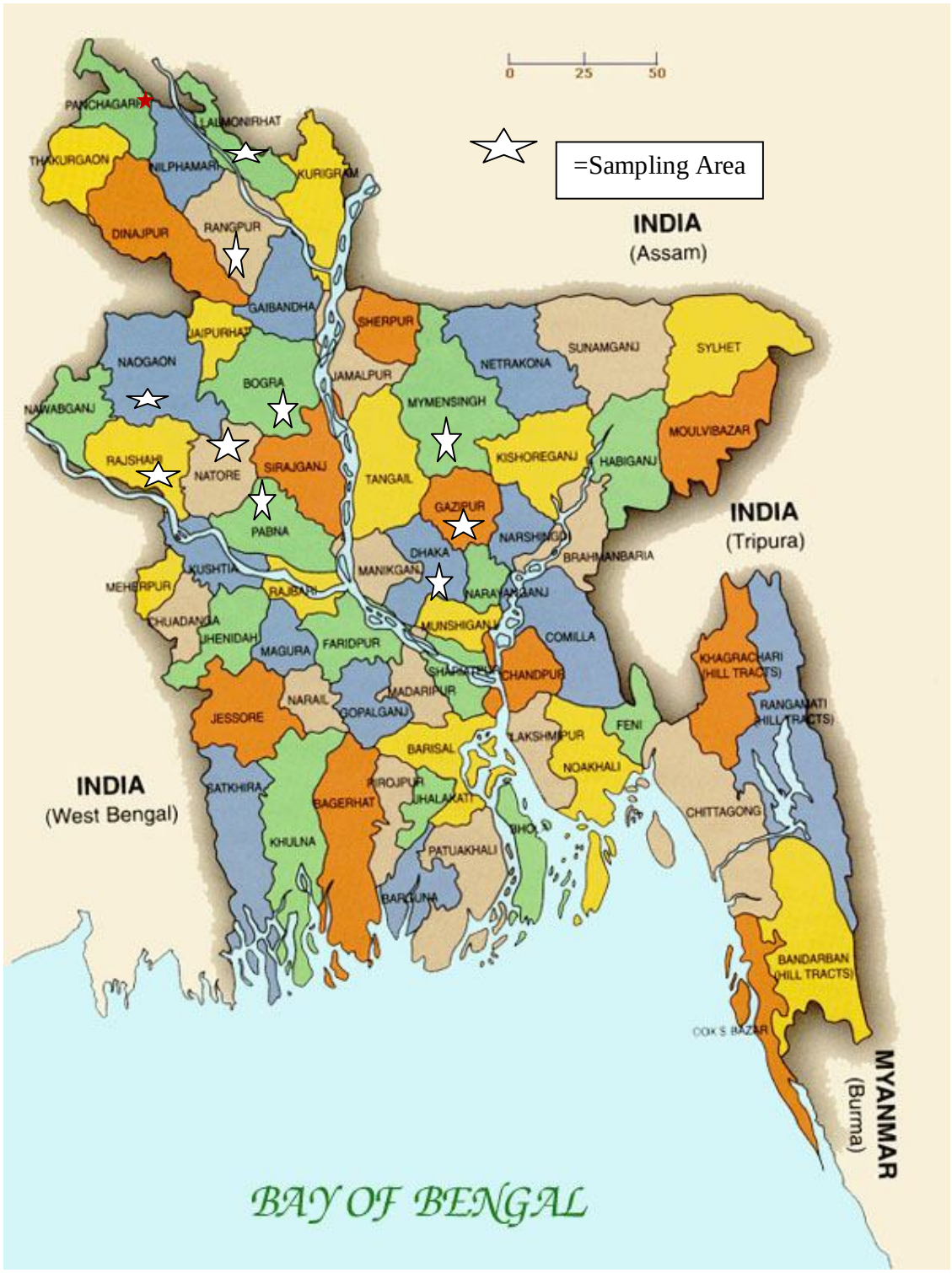


Figure 3. Geographical representation of sampling area

3.4 Designation of collected isolates

The collected isolates were designed as DHA_{Ab}, GAZ_{Ab}, MYM_{Ab} based on their collected location. For example a isolate collected from Dhaka and recognized as first three letter of the area and Ab indicate *A. brassicae*.

Table 1. Designation of collected isolates of *Alternaria* species

District/Thana	Isolates designation	Village/Place
Dhaka (SAU)	DHA_{Ab}	Agronomy field
Gazipur (BARI)	GAZ_{Ab}	Oil Research field
Mymensingh (BAU)	MYM_{Ab}	Horticulture field
Pabna	PAB_{Ab}	Bhabarhat
Rangpur	RAN_{Ab}	Tillalpara
Natore	NAT_{Ab}	Dayarampur
Naogaon	NAO_{Ab}	Kamalpara
Lalmonirhat	LAL_{Ab}	Benupara
Bogra	BOG_{Ab}	Munail
Rajshahi	RAJ_{Ab}	Khorkhori

SAU = Sher-e- Bangla Agricultural University
BAU = Bangladesh Agricultural University
BARI = Bangladesh Agricultural Research Institute

3.5 Isolation, identification, purification and preservation of the pathogen

3.5.1 Preparation of PDA medium

Potato dextrose agar (PDA) were prepared by 200gm potato extract, 1000ml distilled water, 17 gm agar. 20gm dextrose in a conical flask and autoclaved at 121 c under 15 psi for 30 minutes. After autoclaved the media was kept few minutes for cool and added 25-30 drops of lactic acid then poured into sterile petriplates.

3.5.2 Isolation and Identification of *Alternaria* species

The pathogen was isolated by tissue plnting method. The surface area of the clean bench was strilized with 70% eathanol. Then the infected leaf samples were taken into the clean bench and cut into small pices, the cut pices were then strilized in 70% eathanol by dipping for 1minute. Surfaced sterilized leaf pices were taken out with the help of strile forceps and put on sterile distiled water to remove the steriliant for 3 consecutive wash.

After washing the cut pieces were placed on sterilize moist blotter paper (Whatman No. 1) in petriplates (Fig.4) and incubated at 25˚ C for 5-7 days. After incubation the fungal mycelia grew over the infected leaf pieces were transferred on fresh PDA medium (Fig.5) with the help of a sterilize needle and incubated at 25±1˚ C for 7 days. After incubation the fungus mycelia were examined under stereomicroscope (Model: Motic, SMZ-168) & compound microscope (Model: Omano, OMTM-85) for identification of the pathogen (Fig.7). The fungus was identified following the keys of Eills (1971).

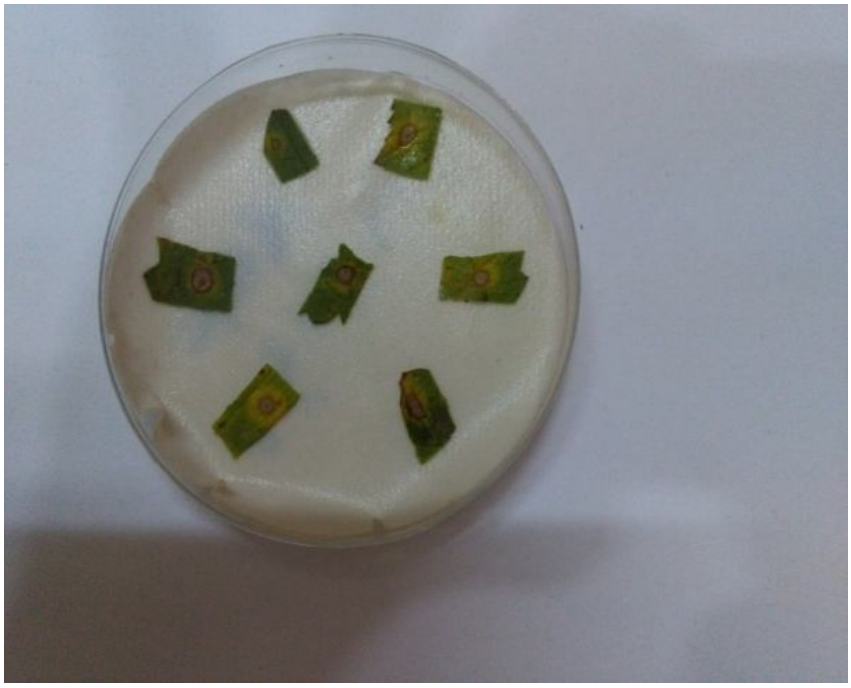


Figure 4. Surface sterilized leaf pieces placed over moistened filter paper

Figure 5. Transfer of mycelia on PDA

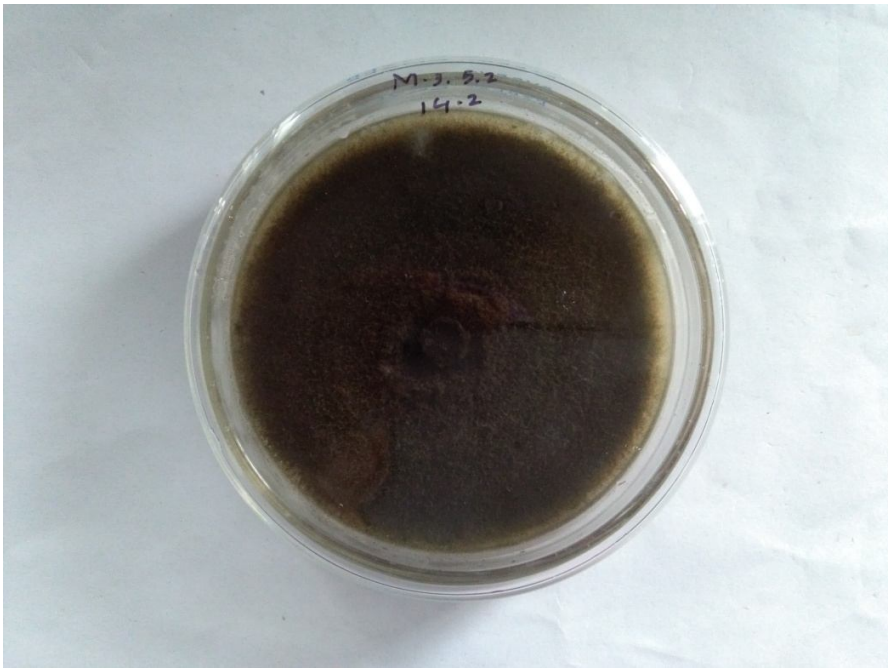


Figure 6. Pure Culture of *Alternaria* species on PDA

Figure 7. Microscopic View of *Alternaria* species at 10X

3.5.3 Purification and preservation of the pathogen

The pure culture of *Alternaria* species from the PDA was transferred to PDA slants and allowed to grow at 25 ± 1^0C for 7 days. After incubation PDA slants were preserved in refrigerator at 4^0C for further study.

3.6 Colony characters of *Alternaria* species

Colony characters in terms of surface colour, colony shape, colony texture, zonation (surface and subsurface) and subsurface colour were studied.

3.7 Morphological variability of *Alternaria* species

All the isolates were studied for morphological variations. In terms of conidia color, shape, size, septation, was observed on PDA medium.The conidial size was measured by using digital microscope (Model: Motic, BA-210) and motic software.

3.8 Effect of culture media and temperature on growth, spore production and time of sporulation

Mycelial discs of 7 days old culture of *Alternaria* species isolates were transferred to the centre of PDA, CDA and PCDA and incubated at 25˚C and 22±1˚C and data were recorded on growth, spore production and time of sporulation. Three replications were maintained for each isolates in a completely randomized design. The colony diameter was recorded on 2, 4, 6, 8, 10, 12, 14 days after inoculation.The spore were calculated by using haemacytometer and digital microscope using the formula of Chauhan and Pandey (1995):

Conidia produced per unit surface = (No. of conidia/ml × Volume of water of suspension)

3.8.1 Preparation of culture medium

3.8.1.1 Preparation of PDA

The procedure of PDA medium preparation is described in section 3.5.1.

3.8.1.2 Preparation of CDA

Carrot dextrose agar were prepared by 200gm carrot extract, 1000ml distilled water, 17 gm agar. 20gm dextrose in a conical flask and autoclaved at 121 c under 15 PSI for 30 minutes. After autoclaved the media was kept few minutes for cool and added 25-30 drops of lactic acid then poured into sterile petriplates.

3.8.1.3 Preparation of Potato-Carrot Dextrose Agar

The combination of Potato-Carrot dextrose agar prepared by 100ml potato+100ml carrot extract, 1000 ml distilled water, 17 gm agar, 20 gm dextrose in a conical flask and autoclaved at 121 c under 15 PSI for 30 minutes. After autoclaved the media was kept few minutes for cool and added 25-30 drops of lactic acid then poured into sterile petriplates.

3.9 Data Analysis

For cultural, morphological and the treatment means the data were statistically analyzed by Dunkan's Multiple Range test (DMRT) with significance level at 5%. (Gomez and Gomez, 1986, Duncan, 1955). The package used for analysis was MSTAT-C version -88, developed by Michigan State University, Agricultural University of Norway (Freed and Scott, 1986).

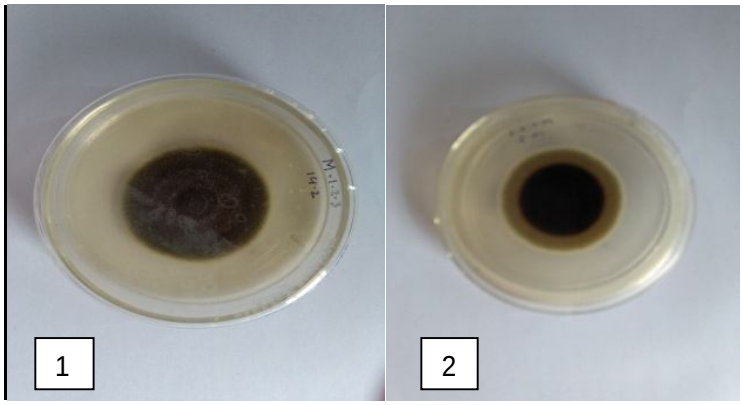
RESULTS

4.1 Colony characters of isolates of *Alternaria* species on PDA

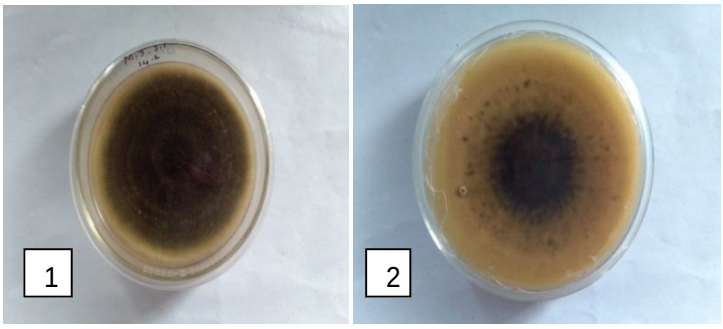
Variation was observed in colony characters of 10 isolates of *Alternaria* species regarding surface colour, shape, texture, zonation and subsurface colour

Table 2. Colony Characters of different isolates of *Alternaria* species on PDA

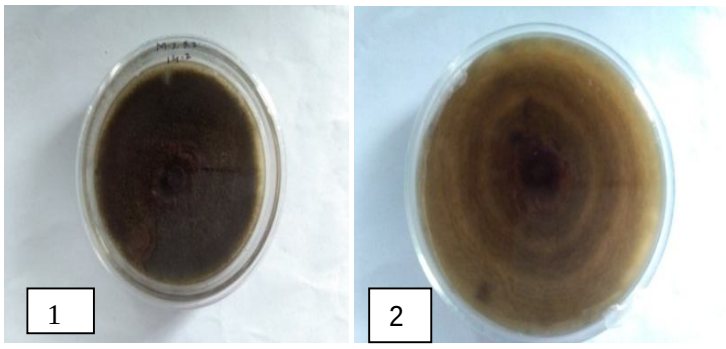
Isolates	Colour		Texture	Colony shape	Zonation	
	Surface	Subsurface			Surface	Subsurface
DHA_{Ab}	Olivacious green	Black center with white surroundings	Cottony	Circular	No zonation	Zonation
GAZ_{Ab}	Olivacious green	Black center with pinkish surroundings	Cottony	Circular	Zonation	Zonation
MYM_{Ab}	Black	Brownish	Velvety	Circular	No zonation	Zonation
PAB_{Ab}	Olivacious green	Black center with pinkish surroundings	Cottony	Circular	No zonation	Zonation
RAN_{Ab}	Black	Black	Cottony	Circular	No zonation	No zonation
NAT_{Ab}	Black	Black center with white surroundings	Cottony	Circular	Zonation	No zonation
NAO_{Ab}	Olivacious green	Greenish black	Velvety	Circular	Zonation	No zonation
LAL_{Ab}	Olivacious green	Light brown	Velvety	Circular	No zonation	Zonation
BOG_{Ab}	Black	Black	Cottony	Circular	No zonation	No zonation
RAJ_{Ab}	Olivacious green	Brownish green with pinkish surroundings	Cottony	Circular	Zonation	Zonation



A. DHA_{Ab}



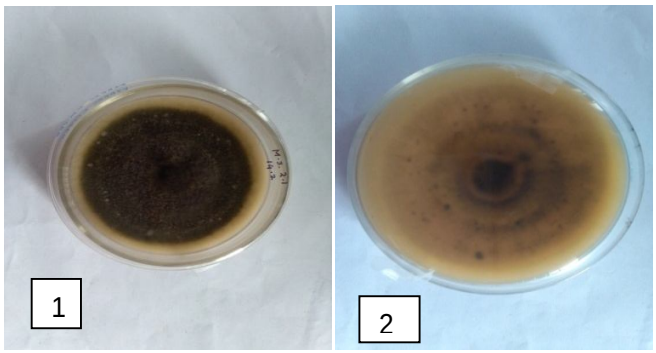
B. GAZ_{Ab}



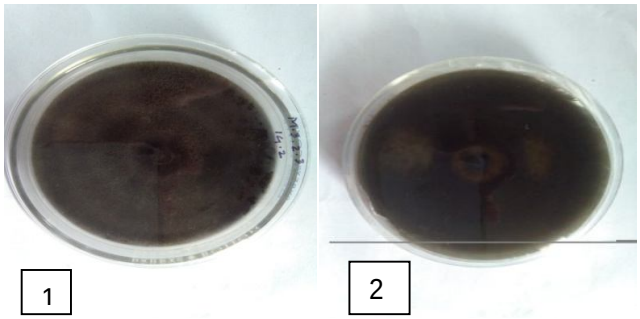
C. MYM_{Ab}

Plate 1. Colony Characters of different isolates of *Alternaria* species on PDA

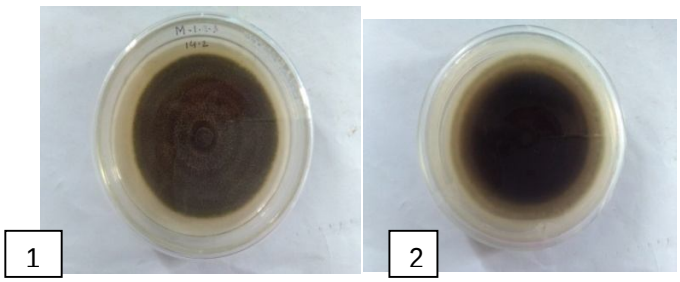
1. Surface Figure
2. Subsurface Figure



D. PAB_{Ab}



E. RAN_{Ab}



F. NAT_{Ab}

Plate 1. Colony Characters of different isolates of *Alternaria* species on PDA (Cont'd.)
1. Surface Figure
2. Subsurface Figure

4.2 Morphological variation of conidia of different isolates of *Alternaria* species.

4.2.1 Size of conidia of *Alternaria* species on PDA medium.

Remarkable variation was observed in length, breadth and beck size of conidia of different isolates of *A. brassicae* on PDA. The length of conidia was varied from 41.56µm to 117.54 µm. The maximum mean length was recorded at MYM_{Ab} that was (113.1 µm). The minimum length (63.63 µm) was recorded at isolates GAZ_{Ab}.

The breadth of conidia of different isolates varied from 10.34 µm to 23.12 µm. The maximum mean breadth (17.36 µm) was recorded at PAB_{Ab} and the minimum mean breadth(20.29 µm) was recorded at LAL_{Ab}.

The beak size of conidia from different isolates varied from 16.78 µm to 72.65 µm. The maximum mean beak length was recorded at PAB_{Ab} that was (43.26 µm) whereas the minimum mean beak length was recorded at GAZ_{Ab} that was (24.84 µm).

Table 3. Size of conidia from different isolates of *Alternaria* species on PDA

Isolate	Length(µm)[1]	Breadth(µm)[1]	Beak(µm)[1]
DHA_{Ab}	88.55 d	18.12 b	28.09 e
GAZ_{Ab}	**63.63 e**	18.09 b	**24.84 e**
MYM_{Ab}	**113.1 a**	18.56 ab	38.76 bc
PAB_{Ab}	103.4 bc	**17.36 b**	**43.26 a**
RAN_{Ab}	99.33 bc	17.90 b	37.19 c
NAT_{Ab}	90.03 d	17.87 b	37.93 c
NAO_{Ab}	87.73 d	20.17 a	25.98 e
LAL_{Ab}	101.5 bc	**20.29 a**	38.95 bc
BOG_{Ab}	97.61 c	18.23 b	41.89 ab
RAJ_{Ab}	104.7 b	19.12 ab	33.23 d
$LSD_{(0.05)}$	6.82	1.82	3.47
CV (%)	4.22	5.75	5.82

[1]Mean of 15 replications for each isolates

Figure 8. Conidial length, breadth and beck (40x)

4.2.2 Conidial characteristics of *Alternaria* species on PDA

Muriform type conidia of *Alternaria* species was observed from all collected isolates.Colour of isolates of *Alternaria* species varied from light brown to deep brown. Isolates NAT_{Ab} and GAZ_{Ab} showed light brown colour, DHA_{Ab}, MYM_{Ab}, LAL_{Ab}, BOG_{Ab} and RAJ_{Ab} isolate showed brown colour whereas PAB_{Ab}. RAN_{Ab} and NAO_{Ab} isolate showed deep brown in colour.

Variation in conidial septation was observed in different isolates of *Alternaria* species. The horizontal septationof each conidium varied from 3-7 to 7-11. The vertical septation of each conidium varied from 0-1 to 2-3. The maximum horizontal septation were observed in isolates BOG_{Ab} (7-11) and the minimum septation was observed in isolates GAZ_{Ab}.The maximum vertical (2-3) septation of each conidium observed in isolates MYM_{Ab}, RAN_{Ab} and NAT_{Ab} and the minimum vertical (0-1) septation observed in isolate DHA_{Ab}.

Table 4. Conidial characteristics of *Alternaria* species

Isolates	**Conidial type**	**Conidial Colour**	**Conidial septation (Range)**	
			Horizontal	Vertical
DHA_{Ab}	Muriform	Brown	5-9	0-1
GAZ_{Ab}	Muriform	Light brown	3-7	0-2
MYM_{Ab}	Muriform	Brown	5-7	2-3
PAB_{Ab}	Muriform	Deep Brown	5-7	1-2
RAN_{Ab}	Muriform	Deep Brown	5-7	2-3
NAT_{Ab}	Muriform	Light brown	7-11	2-3
NAO_{Ab}	Muriform	Deep Brown	5-8	1-3
LAL_{Ab}	Muriform	Brown	7-9	1-3
BOG_{Ab}	Muriform	Brown	7-11	0-3
RAJ_{Ab}	Muriform	Brown	5-9	1-2

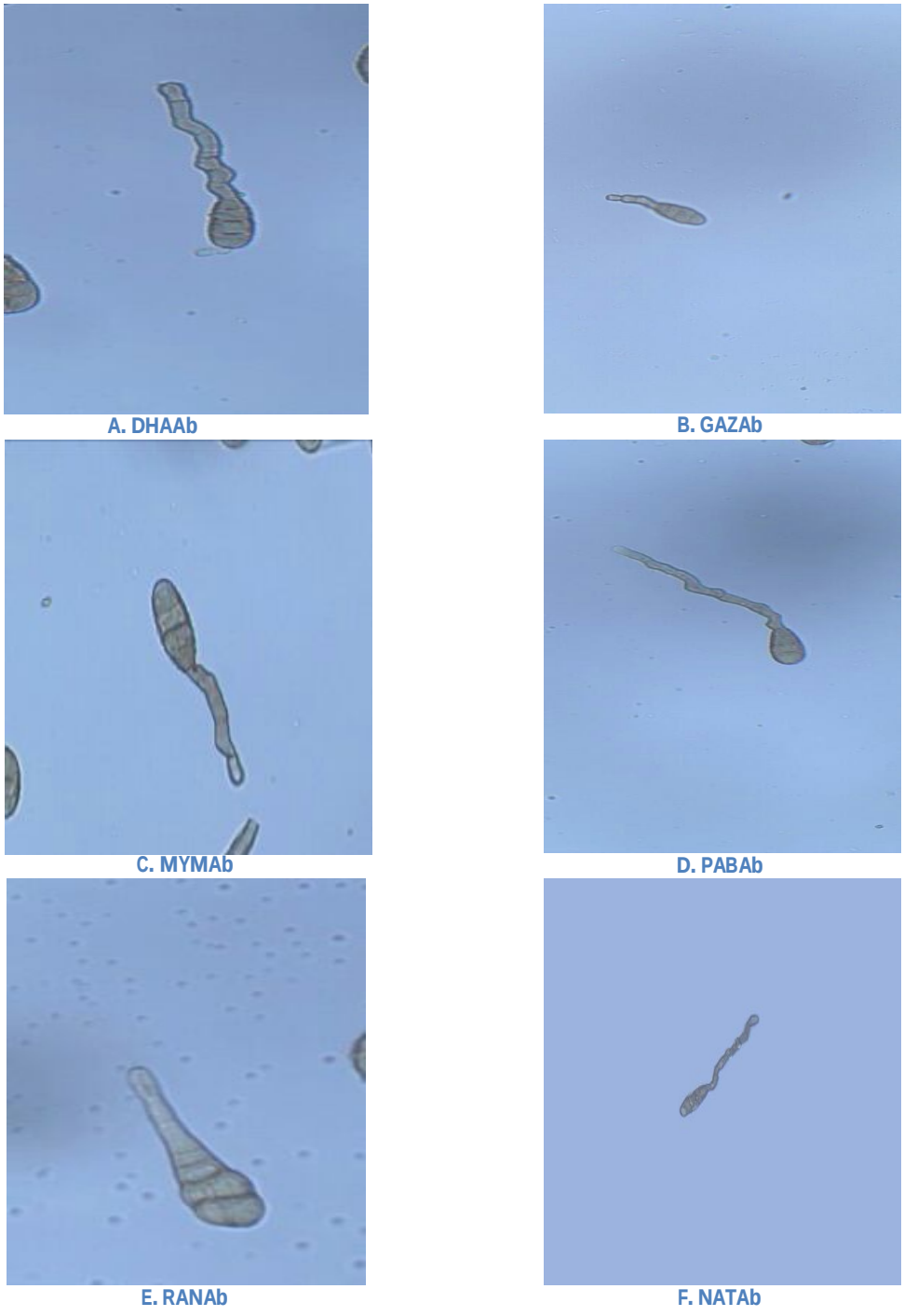
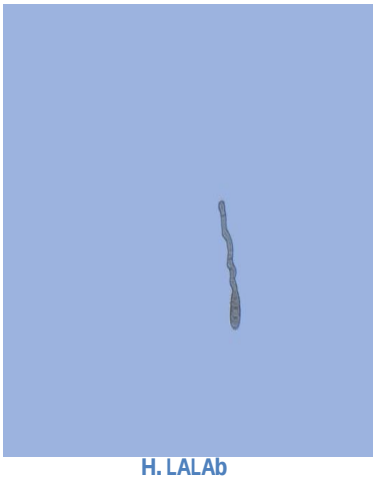
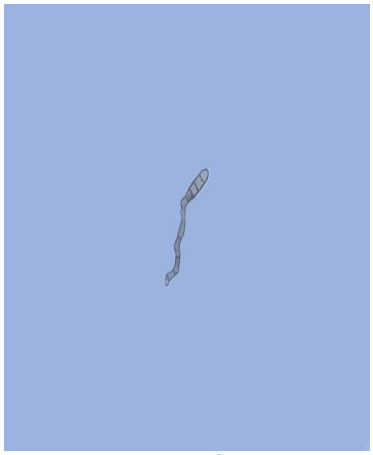


Plate 2: Condial characteristics of *Alternaria* species

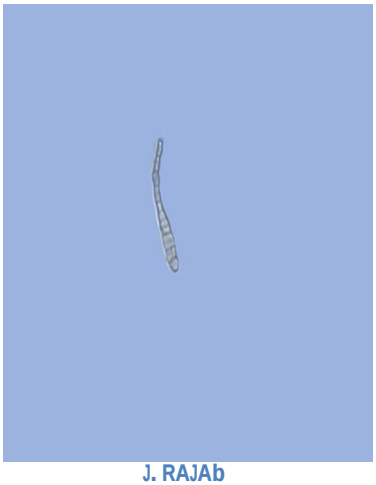
G. NAOAb



H. LALAb



I. BOGAb



J. RAJAb

Plate 2: Conidial characteristics of *Alternaria* species (Cont'd.)

4.3 Cultural variability of *Alternaria* species

4.3.1 Radial mycelial growth of 10 isolates of *Alternaria* species at 25˚C on different media

4.3.1.1 Radial mycelial growth of 10 isolates of *Alternaria* species on PDA

Radial mycelial growth of different isolates of *Alternaria* species significantly varied on PDA (Table and Plate). After 2 days of inoculation the maximum radial mycelial growth of *Alternaria* species was observed in DHA_{Ab} (30.50 mm), followed by PAB_{Ab} (27.00 mm). The minimum radial mycelial growth was recorded in NAO_{Ab} (14.67 mm) which was statistically similar to GAZ_{Ab} (17.33 mm).

After 4^{th} day, 6^{th} day, 8^{th} day, 10^{th} day and 12^{th} day of inoculation the maximum radial mycelial growth of *Alternaria* species were recorded in DHA_{Ab} which were 48.33 mm, 64.00 mm, 79.93 mm, 89.33 mm and 90.00 mm, respectively and the minimum radial mycelial growth were recorded in NAO_{Ab} which were 33.50 mm, 47.33 mm, 58.33 mm, 67.00 mm and 73.33 mm respectively.

After 14 days of inoculation the maximum radial mycelial growth of *Alternaria* species was measured in DHA_{Ab} which was 90.00 mm, followed by PAB_{Ab} (88.33 mm) whereas the minimum radial mycelial growth was recorded in NAO_{Ab} (76.67 mm) which was statistically similar to MYM_{Ab} (79.67 mm).

Table 5. Radial mycelial growth of different isolates of *Alternaria* species at different days after incubation on PDA

Isolate	2thDay	4th Day	6th Day	8th Day	10th Day	12th Day	14th Day
DHA_{Ab}	**30.50 a**	**48.33 a**	**64.00 a**	**79.93 a**	**89.33 a**	**90.00 a**	**90.00 a**
GAZ_{Ab}	17.33 f	38.00 d	51.67 d-f	69.00 bc	76.33 cd	79.33 b-d	80.33 cd
MYM_{Ab}	20.33 e	41.83 b-d	50.50 ef	67.67 c	73.00 de	77.00 cd	79.67 cd
PAB_{Ab}	27.00 b	42.33 bc	59.37 a-c	73.33 a-c	81.33 bc	82.67 a-c	83.00 a-d
RAN_{Ab}	23.33 d	45.67 ab	55.67 cd	70.57 bc	75.50 cd	78.33 b-d	82.67 b-d
NAT_{Ab}	17.67 f	40.67 cd	56.33 b-d	73.67 a-c	79.13 b-d	84.67 a-c	85.00 a-c
NAO_{Ab}	**14.67 g**	**33.50 e**	**47.33 f**	**58.33 d**	**67.00 e**	**73.33 d**	**76.67 d**
LAL_{Ab}	21.00 e	41.67 cd	54.67 c-e	76.67 ab	84.67 ab	82.67 a-c	83.00 a-d
BOG_{Ab}	25.67 bc	43.50 bc	58.50 bc	75.83 ab	80.33 b-d	83.33 a-c	85.00 a-c
RAJ_{Ab}	24.33 cd	40.83 cd	61.00 ab	74.93 a-c	81.00 bc	86.33 ab	88.33 ab
$LSD_{(0.05)}$	1.97	3.87	5.17	7.77	7.93	8.54	7.10
CV (%)	5.21	5.46	5.42	6.34	5.91	6.14	5.00

4.3.1.2 Radial mycelial growth of 10 isolates of *Alternaria* species on CDA

After 2 days of inoculation the maximum radial mycelial growth of *Alternaria* species was recorded 29.67 mm in NAT_{Ab}, followed by RAJ_{Ab} (28.67 mm). The minimum radial mycelial growth was recorded in GAZ_{Ab} (17.67 mm) which was statistically similar to MYM_{Ab} (19.17 mm).

After 4^{th} days, 6^{th} days, 8^{th} days, 10^{th} day and 12^{th} days of inoculation the maximum radial mycelial growth of *Alternaria* species were measured in NAT_{Ab} which was 49.17 mm, 65.33 mm, 82.33 mm, 87.00 mm and 90.00 mm respectively and the minimum radial mycelial growth were recorded in GAZ_{Ab} 26.33 mm, 42.33 mm, 54.33 mm, 73.33 mm and 77.67 mm respectively.

After 14 days of inoculation the maximum radial mycelial growth of *Alternaria* species was measured in NAT_{Ab} **(**90.00 mm), which was statically similar to RAJ_{Ab} (89.33 mm). The minimum radial mycelial growth was recorded in GAZ_{Ab} (83.67mm) which was statistically similar to MYM_{Ab} (85.33 mm).

Table 6. Radial mycelial growth of different isolates of *Alternaria* species at different days after incubation on CDA

Isolate	2th Day	4th Day	6th Day	8th Day	10th Day	12th Day	14th Day
DHA_{Ab}	25.83 c	42.67 b-d	53.67 cd	64.83 ef	77.67 bc	80.00 de	86.67 ab
GAZ_{Ab}	**17.67 e**	**26.33 f**	**42.33 f**	**54.33 g**	**73.33 c**	**77.67 e**	**83.67 b**
MYM_{Ab}	19.17 e	33.17 e	48.00 e	61.50 f	82.00 ab	83.67 b-d	85.33 ab
PAB_{Ab}	26.67 bc	43.00 b-d	58.67 b-d	72.67 b-d	81.67 ab	85.33 a-d	87.33 ab
RAN_{Ab}	22.00 d	38.83 d	53.17 de	69.77 de	81.67 ab	82.67 b-e	88.33 ab
NAT_{Ab}	**29.67 a**	**49.17 a**	**65.33 a**	**82.33 a**	**87.00 a**	**90.00 a**	**90.00 a**
NAO_{Ab}	26.50 bc	42.67 b-d	58.83 bc	70.67 c-e	75.67 bc	85.33 a-d	87.67 ab
LAL_{Ab}	26.00 c	42.00 cd	60.67 ab	75.33 b-d	76.67 bc	81.33 c-e	86.33 ab
BOG_{Ab}	27.33 a-c	46.50 ab	61.67 ab	76.33 a-c	82.33 ab	86.67 a-c	88.67 ab
RAJ_{Ab}	28.67 ab	44.33 bc	63.33 ab	78.33 ab	85.33 a	88.33 ab	89.33 ab
$LSD_{(0.05)}$	2.36	4.35	5.55	6.49	7.63	5.82	6.28
CV (%)	5.5	6.24	5.76	5.4	5.58	4.06	4.22

4.3.1.3 Radial mycelial growth of 10 isolates of *Alternaria* species on PCDA

After 2 days of inoculation the maximum radial mycelial growth of *Alternaria* species was measured in RAJ_{Ab} on PCDA medium which was **(**33.17 mm) that was statically similar to RAN_{Ab} (31.83 mm). The minimum radial mycelial growth was recorded in NAO_{Ab} (20.33 mm) which was statistically similar to MYM_{Ab} (23.33 mm).

After 4^{th} days, 6^{th} days, 8^{th} days, 10^{th} days and 12^{th} days of inoculation the maximum radial mycelial growth of *Alternaria* species 54.83 mm, 68.17 mm, 80.27 mm, 88.33 mm and 90.00 mm were measured in RAJ_{Ab} and the minimum radial mycelial growth were recorded in NAO_{Ab} 33.83 mm, 48.33 mm, 64.67mm, 70.67 mm and 74.67 mm, respectively.

After 14 days of inoculation the maximum radial mycelial growth of *Alternaria* species was recorded in RAJ_{Ab} **(**90.00 mm), which was statically similar to BOG_{Ab} and NAT_{Ab}(90.00 mm). The minimum radial mycelial growth was recorded in NAO_{Ab} (79.00 mm) preceded by MYM_{Ab} (85.33 mm).

Table 7.Radial mycelial growth of different isolates of *Alternaria* species at different days after incubation on PCDA

Isolate	2th Day	4th Day	6th Day	8th Day	10th Day	12th Day	14th Day
DHA_{Ab}	26.67 bc	45.20 cd	61.33 b	75.67 ab	85.00 ab	88.00 a-c	89.00 ab
GAZ_{Ab}	26.67 bc	45.20 cd	62.30 ab	78.27 a	84.20 ab	87.67 a-c	88.00 ab
MYM_{Ab}	23.33 cd	42.33 de	52.33 c	69.33 bc	77.00 bc	81.67 c	85.33 c
PAB_{Ab}	29.67 ab	50.00 a-c	63.50 ab	78.33 a	85.33 ab	88.67 ab	89.33 ab
RAN_{Ab}	31.83 a	46.33 b-d	63.00 ab	76.00 a	83.33 bc	86.33 a	89.67 ab
NAT_{Ab}	24.33 c	39.67 e	64.83 ab	76.10 a	84.83 ab	89.67 ab	**90.00 a**
NAO_{Ab}	**20.33 d**	**33.83** f	**48.33 c**	**64.67 c**	**70.67 c**	**74.67 d**	**79.00 d**
LAL_{Ab}	31.50 a	46.33 b-d	65.33 ab	78.67 a	83.00 ab	85.33 a-c	87.33 bc
BOG_{Ab}	31.00 a	50.67 ab	66.33 ab	79.00 a	84.33 ab	88.67 ab	**90.00 a**
RAJ_{Ab}	**33.17 a**	**54.83 a**	**68.17 a**	**80.27 a**	**88.33 a**	**90.00 a**	**90.00 a**
$LSD_{(0.05)}$	3.62	5.19	6.05	6.64	8.86	6.41	2.66
CV (%)	7.65	6.71	5.77	5.16	6.27	4.39	1.78

4.4 Effect of different temperature on growth of *Alternaria* species on different media at 14th DAI

4.4.1 Effect of temperature on growth of *Alternaria* species on PDA medium at 14th DAI

Significant variation was observed among the isolates on PDA medium at different temperature (Fig.9). The isolate DHA_{Ab} showed the highest growth at 25˚C but in case of at 22±1˚C temperature the growth reduced significantly. The minimum growth was observed in isolate NAO_{Ab} at both temperature.

Isolate MYM_{Ab} showed higher growth at 22±1˚C but lower growth at 25˚C. In isolates GAZ_{Ab}, PAB_{Ab}, RAN_{Ab}, NAT_{Ab}, LAL_{Ab}, BOG_{Ab} and RAJ_{Ab} no significant variation were observed at both temperature.

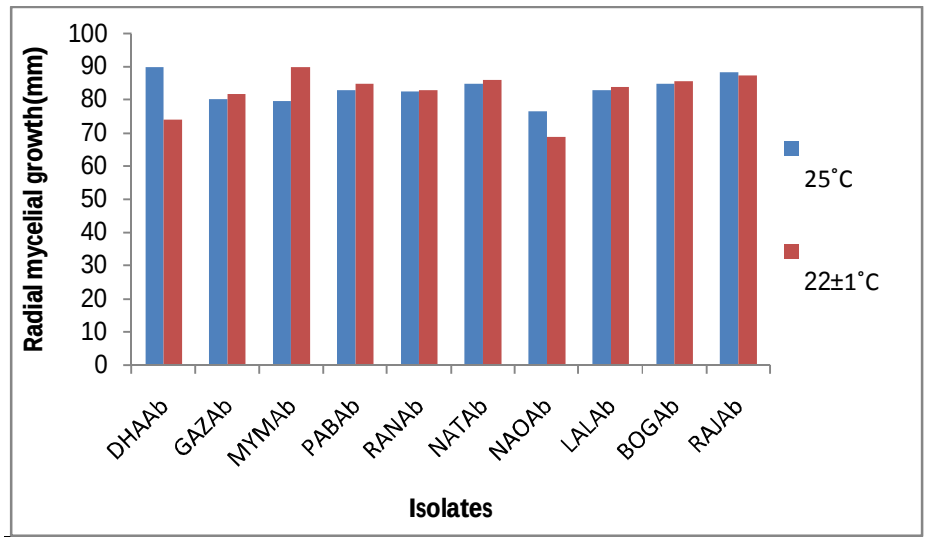


Figure 9. Effect of temperature on growth of *Alternaria* species on PDA medium at 14th DAI

4.4.2 Effect of temperature on growth of *Alternaria* species on CDA medium at 14th DAI

No significant variation was observed in the radial mycial growth among the isolates at 25°C and 22±1°C temperature on CDA (Fig.10).

In case of NAO_{Ab} radial mycial growth were minimum at both temperature but at 22±1°C temperature it was lowest than 25°C temperature.

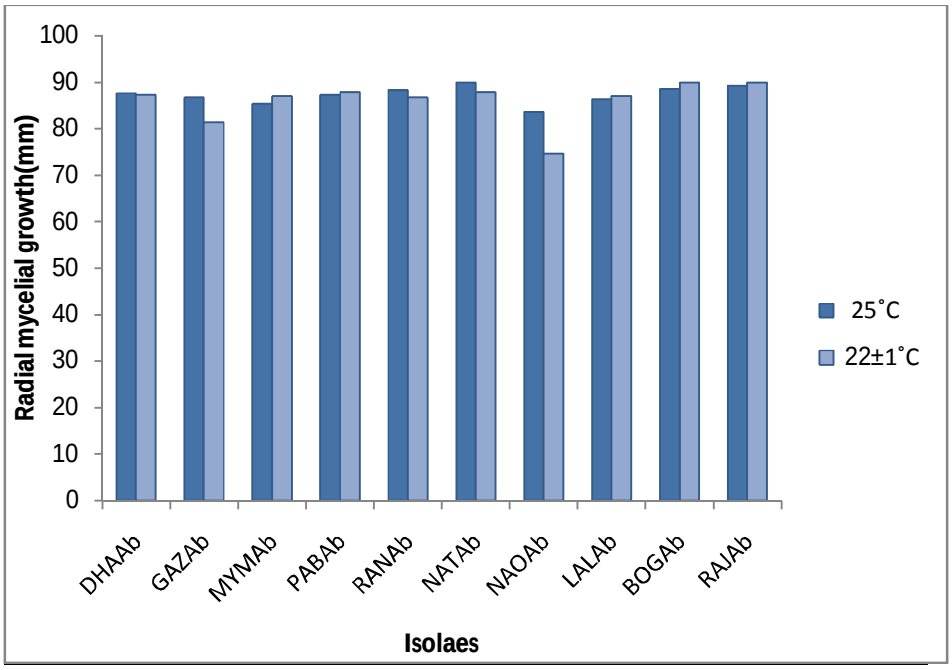


Figure 10. Effect of temperature on growth of *Alternaria* species on CDA medium at 14th DAI.

4.4.3 Effect of temperature on growth of *Alternaria* species on PCDA at 14th DAI

Significant variation was observed on mycial growth among the isolates (Fig.11). Isolates DHA_{Ab} and GAZ_{Ab} higher growth at 22±1°C but low at 25°C.

Isolates MYM_{Ab}, LAL_{Ab} BOG_{Ab} and RAJ_{Ab} no significant variation were observed onmycial growth at both temperature.

In case of isolates $PAB_{Ab,}$ RAN_{Ab} and NAT_{Ab} 25°C temperature found suitable for mycelial growth compare to 22±1°C temperature. Isolate NAO_{Ab} had the lowest mycelial growth at 25°C temperature but comparatively higher at 22±1°C temperature.

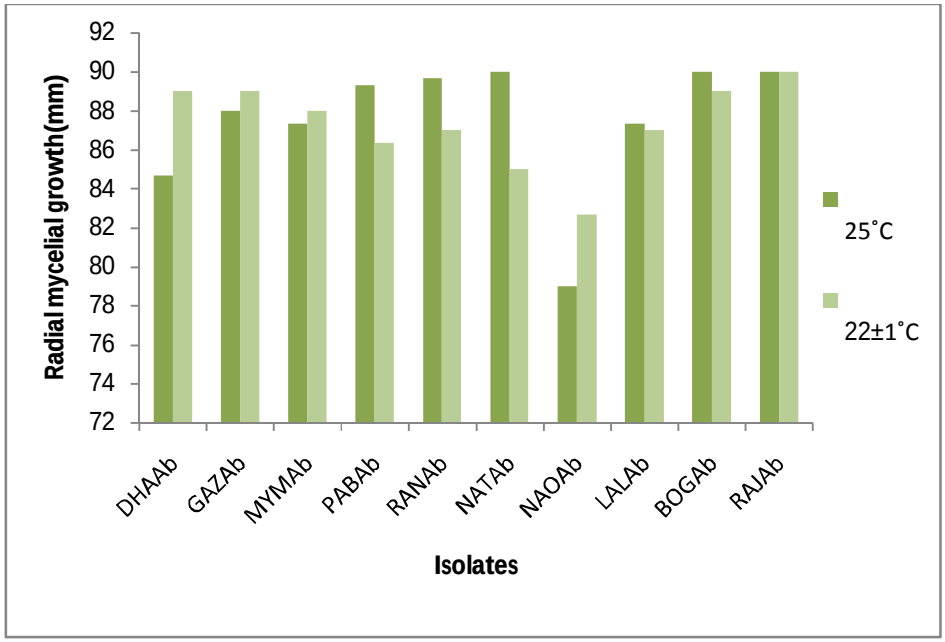


Figure 11. Effect of temperature on growth of *A. brassicae* on PCDA at 14th DAI.

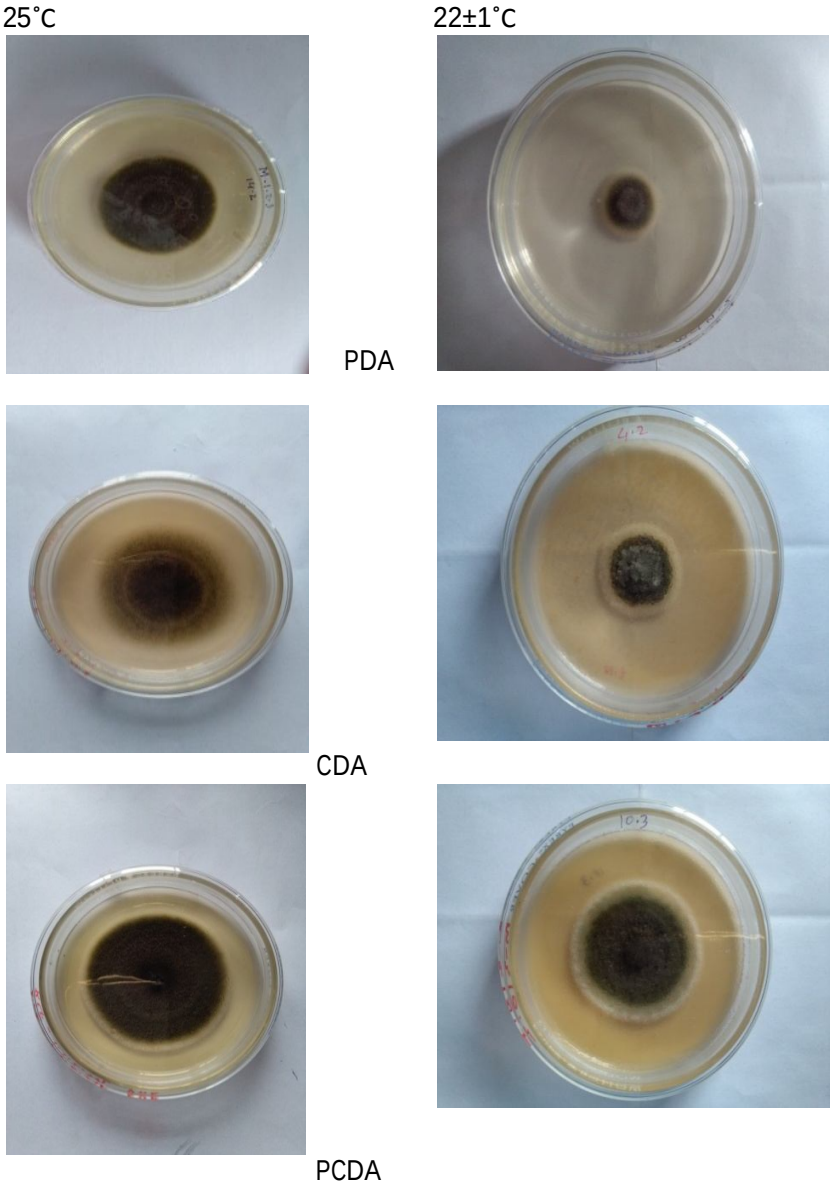


Plate 3. Myceial growth of *Alternaria* species at different temperature on different media at 6thDAI

4.5 Spore production

4.5.1 Spore production of different isolates of *Alternaria* species at different temperature on different media.

4.5.1.1 Spore production of different isolates of *Alternaria* species on PDA medium

Spore production was varied among different isolates on PDA (Table.8). At 25˚C the highest no of spore was count at DHA_{Ab} isolates which was 45.32×10^6/ ml followed by RAJ_{Ab} (39.92×10^6/ ml). The lowest no of spore were count in isolates NAO_{Ab} 6.510×10^6/ ml preceded by LAL_{Ab} **(**15.31×10^6/ ml). At 22±1˚C The highest no of spore count at isolates MYM_{Ab} which was 27.97×10^6/ ml followed by PAB_{Ab} (23.72×10^6/ ml). The lowest no of spore were count in isolates RAN_{Ab} 5.35×10^6/ ml preceded by LAL_{Ab} **(**10.20×10^6/ ml).

4.5.1.2 Spore production of different isolates of *Alternaria* species on CDA

Spore production varied on different isolates on CDA (Table.8). At 25˚C The highest no of spore count at NAT_{Ab} isolates which was 50.25×10^6/ ml followed by RAJ_{Ab} (37.73×10^6/ ml). The lowest no of spore were count in isolates DHA_{Ab} 11.48×10^6/ ml preceded by LAL_{Ab} **(**16.90×10^6/ ml). At 22±1˚C The highest no of spore count at RAJ_{Ab} isolates which was 37.12×10^6/ ml followed by BOG_{Ab} (33.00×10^6/ ml).The lowest no of spore were count in isolates $PAB_{Ab}10.82 \times10^6$/ ml preceded by DHA_{Ab} **(**14.07×10^6/ ml).

4.5.1.3 Spore production of different isolates of *Alternaria* species on PCDA

Spore production varied on different isolates on PCDA(Table.8) At 25˚C the highest no of spore count at RAJ_{Ab} isolates which was 59.79 $\times 10^6$/10 ml followed by BOG_{Ab} (43.33 $\times$ 10^6/ ml).The lowest no of spore were count in isolates NAO_{Ab}

(14.17 $\times 10^6$/ ml**)** preceded by LAL_{Ab} **(**25.42 $\times 10^6$/ ml). At 22±1˚C the highest no of spore count at RAJ_{Ab} isolates which was 45.32 $\times 10^6$/ ml followed by BOG_{Ab} (35.42 $\times$ 10^6/ ml).The lowest no of spore were count in isolates NAO_{Ab} (17.33 $\times 10^6$/ ml**)** preceded by DHA_{Ab} **(**26.32 $\times 10^6$/ ml).

Table 8.No. of spores of different isolates of *Alternaria* species at different temperature and media

Isolate	25˚C			22±1˚C		
	PDA*	CDA*	PCDA*	PDA*	CDA*	PCDA*
DHA_{Ab}	**45.32 a**	**11.48 h**	29.58 ef	8.52 e	14.07 de	26.32 e
GAZ_{Ab}	22.15 e	20.82 ef	25.97 fg	13.57 d	17.17 d	30.15 cd
MYM_{Ab}	32.90 c	25.42 d	38.23 c	**27.97 a**	31.00 bc	31.63 c
PAB_{Ab}	30.61 cd	19.23 fg	32.67 de	23.72 b	**10.82 e**	31.33 c
RAN_{Ab}	28.29 d	23.58 de	35.33 cd	**5.35 f**	28.67 bc	31.92 bc
NAT_{Ab}	31.15 cd	**50.25 a**	28.00 fg	23.08 b	26.23 c	30.65 cd
NAO_{Ab}	**6.510 g**	22.83 de	**14.17 h**	20.40 c	29.18 bc	**17.33 f**
LAL_{Ab}	15.31 f	16.90 g	25.42 g	10.20 e	15.8 d	27.50 de
BOG_{Ab}	38.57 b	33.87 c	43.33 b	22.45 bc	33.00 ab	35.42 b
RAJ_{Ab}	39.92 b	37.73 b	**59.79 a**	23.07 b	**37.12 a**	**45.32 a**
$LSD_{(0.05)}$	3.18	3.36	3.73	2.28	4.84	3.60
CV (%)	6.42	7.52	6.59	7.52	11.69	6.87

*PDA=Potato Dextrose Agar;CDA=Carrot Dextrose Agar;PCDA=Potato Carrot Dextrose Agar

4.6.1 Effect of different temperature on spore production of *Alternaria* species on PDA medium.

Significant variations were observed in case of spore production on different temperature (Fig.12).All isolates produced higher no of spore at 25˚C and lower no of spore at 22±1˚C temperature .

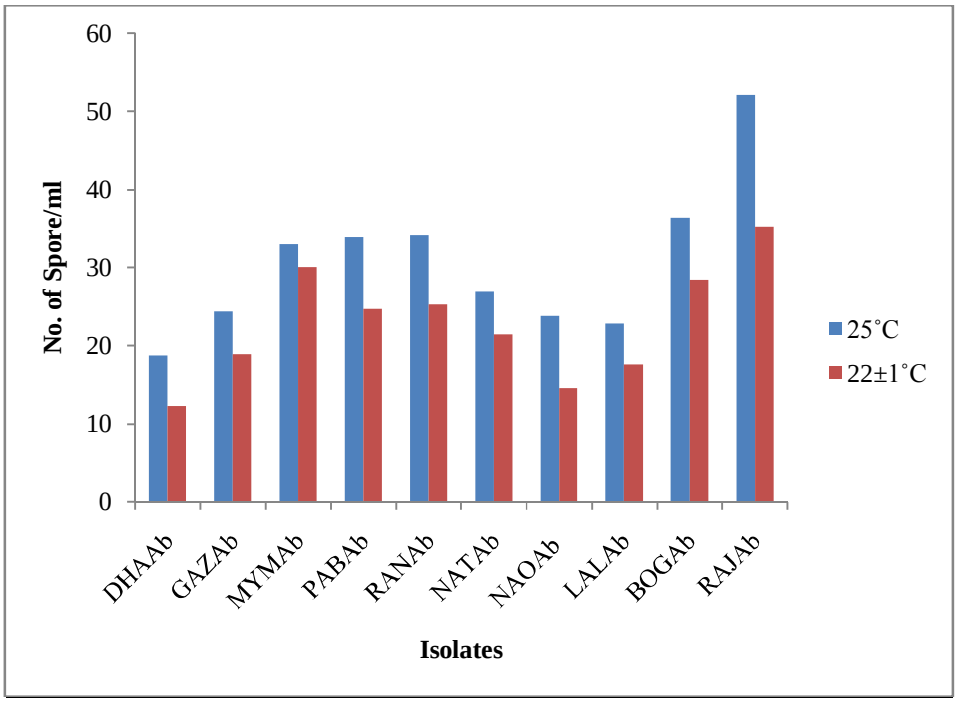
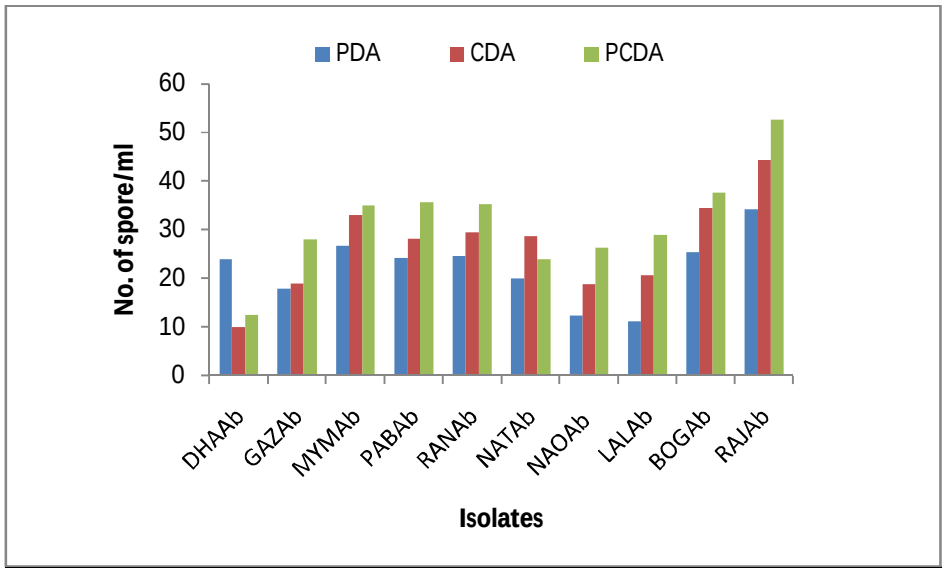


Figure 12. Effect of different temperature on no. of spores of different isolates of *Alternaria* species on PDA medium.

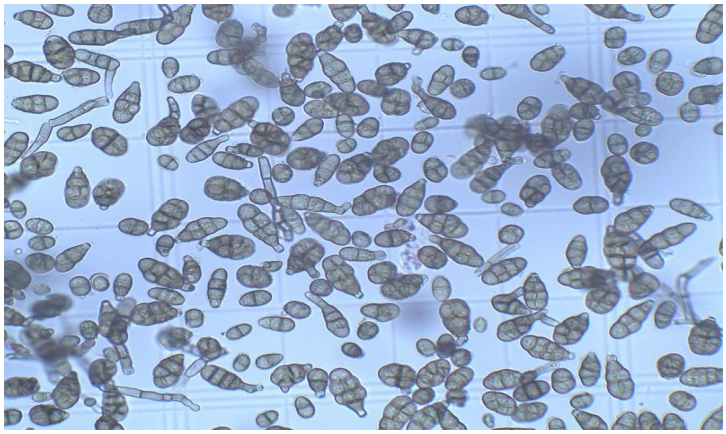
4.6.2 Effect of different media on spore production of *Alternaria* species at 25˚C.

Significant variations were observed on spore production on different media (Fig-13). All isolates produced higher no of spore on PCDA medium except isolate DHA_{Ab} it produced maximum spore on PDA medium. On CDA medium all isolates produced moderate spore and on PDA medium lower spore recorded.

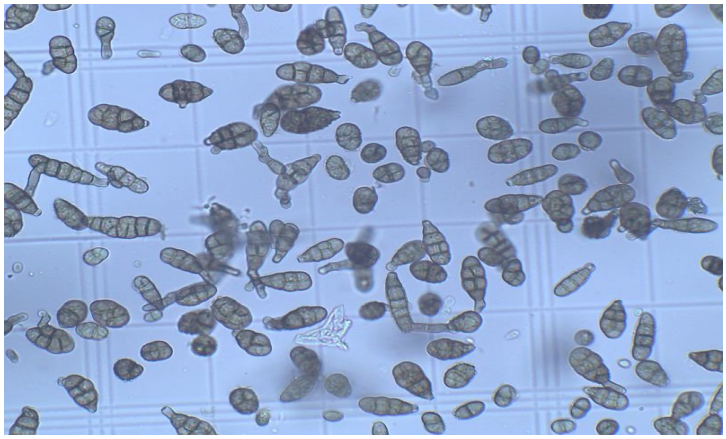


PDA=Potato Dextrose Agar
CDA=Carrot Dextrose Agar
PCDA=Potato Carrot Dextrose Agar

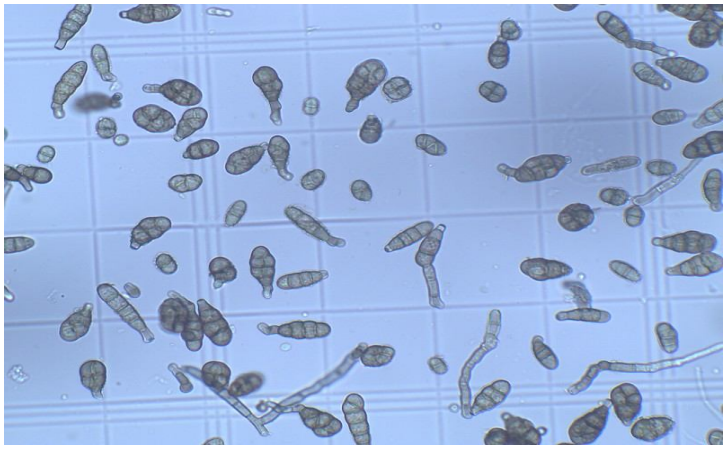
Figure 13. Effect of different media on no. of spores of different isolates of *Alternaria* species at 25˚C.



A. PCDA

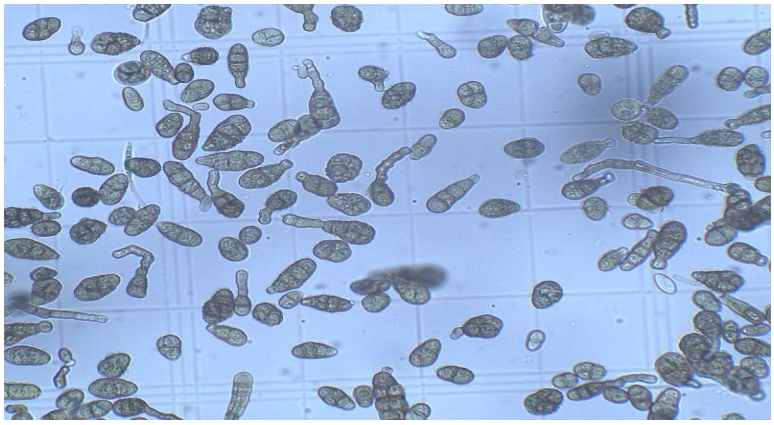


B. CDA

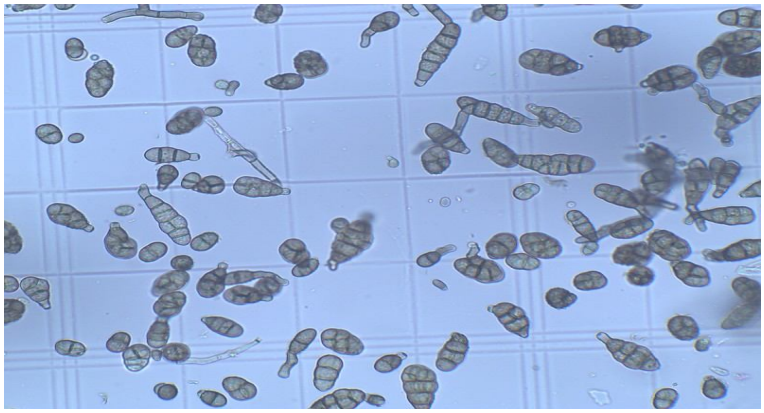


C. PDA

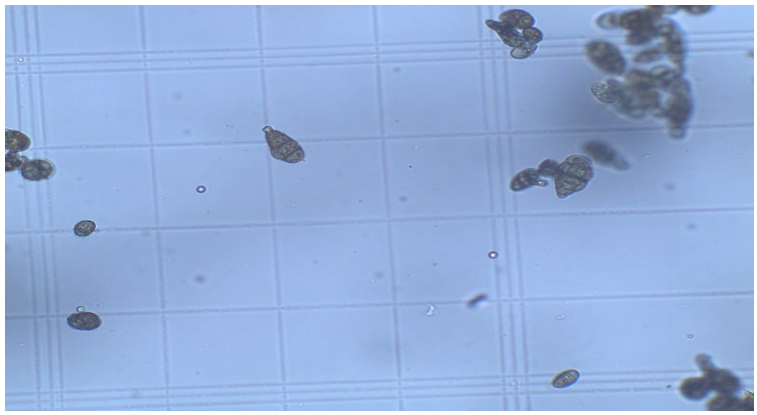
Plate 5. Spore production of *Alternaria* species at 25˚C counted using hemocytometer under compound microscope (40x)



A. PCDA



B. CDA



C. PDA

Plate 6. Spore production of *Alternaria* species at at 22±1˚C counted using hemocytometer under compound microscope(40x)

4.7 Sporulation time of *Alternaria* species

4.7.1 Sporulation time of different isolates on different temperature and on different media

4.7.1.1 Sporulation time on PDA medium

At 25˚C On PDA media sporulation time varied from 7-11 days (Table.9). The minimum days required at isolates RAN_{Ab} which was 7 days followed by BOG_{Ab}. The maximum days required at isolates NAO_{Ab} 11 days followed by MYM_{Ab}. At 22±1˚C Sporulation time on PDA media varied from 8-11 days. The minimum days required for sporulation in isolates MYM_{Ab} (8 days) and maximum days required for isolates PAB_{Ab} (11days).

4.7.1.2 Sporulation time on CDA medium

At 25˚C On CDA media sporulation time varied from 6-9 days (Table.9).The minimum days required at isolates RAJ_{Ab}which was 6 days preceded by BOG_{Ab} and NAT_{Ab}.The maximum days required at isolates MYM_{Ab} 9 days. At 22±1˚C Sporulation time on CDA media varied from 7-10 days.The minimum days required for sporulation in isolates LAL_{Ab} (7 days) and maximum days required for isolates NAO_{Ab} (10 days).

4.7.1.3 Sporulation time on PCDA

At 25˚C In case of PCDA media sporulation time varied from 4-7 days (Table.9). The minimum days required at isolates RAJ_{Ab}which was 4days.The maximum days required at isolates PAB_{Ab} which was 7 days. At 22±1˚C Sporulation time on PCDA media varied from 6-9 days. The minimum days required for sporulation in isolates RAJ_{Ab} (6 days) and maximum days required for isolates RAN_{Ab} (10 days).

Table 9. Time of Sporulation among different isolates of *Alternaria* species at different temperature on different media

Sporulation Time						
Isolate	25˚C			22±1˚C		
	PDA*	CDA*	PCDA*	PDA*	CDA*	PCDA*
DHA_{Ab}	10.00 b-d	7.00 b-d	5.00 cd	11.00 a-c	10.00 ab	9.00 a
GAZ_{Ab}	10.00 b-d	8.00 b	5.00 cd	10.00 bc	10.00 ab	8.00 cd
MYM_{Ab}	11.00 ab	**9.00 a**	5.00 cd	**8.00 d**	9.00 cd	8.00 cd
PAB_{Ab}	10.33 bc	8.00 b	**7.00 a**	**12.00 a**	9.00 cd	8.00 cd
RAN_{Ab}	**7.00 e**	8.00 b	6.00 b	10.00 bc	8.00 de	**9.00 a**
NAT_{Ab}	10.00 b-d	7.00 de	5.00 cd	11.00 a-c	9.00 cd	7.00 d
NAO_{Ab}	**12.00 a**	8.00 bc	5,00 cd	11.00 a-c	**11.00 a**	8.00 cd
LAL_{Ab}	9.00 cd	7.00 cd	5.00cd	11.00 a-c	**7.00 e**	8.00 cd
BOG_{Ab}	8.00 e	7.00 cd	**4.00 e**	10.00 bc	8.00 de	7.00 d
RAJ_{Ab}	9.00 d	**6.00 e**	**4.00 e**	11.00 a-c	10.00 ab	**6.00 e**
$LSD_{(0.05)}$	1.01	0.88	0.62	1.16	0.92	0.95
CV (%)	6.16	6.92	7.21	6.51	6	7.12

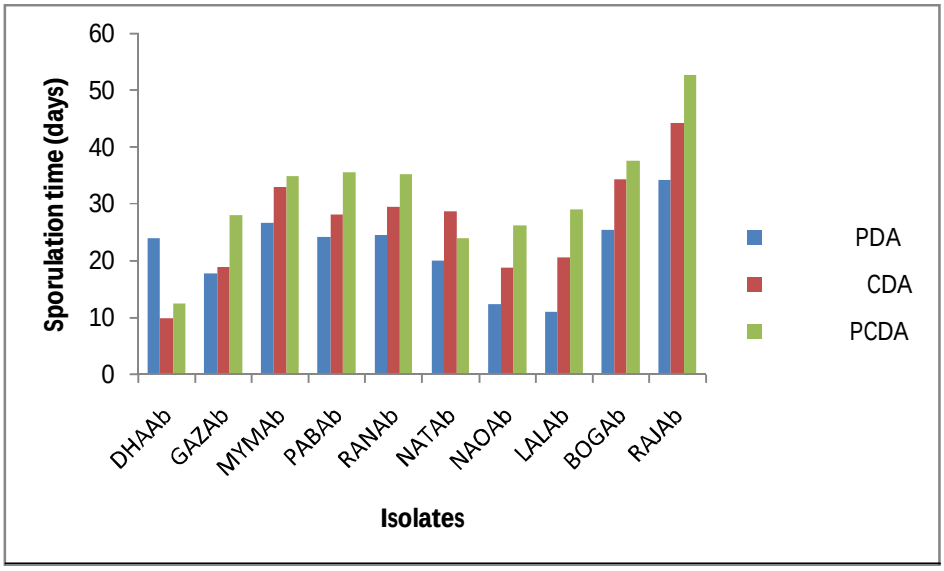
*PDA=Potato Dextrose Agar;CDA=Carrot Dextrose Agar;PCDA=Potato Carrot Dextrose Agar

4.8.1 Effect of different media on sporulation time of different isolates of *Alternaria* species.

Variation were observed in spore production on different media. Except isolate PAB_{Ab} all isolate required less time for sporulation on PCDA. On CDA moderate time required. On PDA maximum time required for sporulation.(Fig.14)

4.8.2 Effect of different temperature on sporulation time of different isolates of *Alternaria* species.

All isolates were took less time for sporulation at 25˚C and maximum sporulation time required at 22±1˚C (Fig.15)



PDA=Potato Dextrose Agar
CDA=Carrot Dextrose Agar
PCDA= Potato Carrot Dextrose Agar

Figure 14. Effect of different media on sporulation time of different isolates of *Alternaria* species .

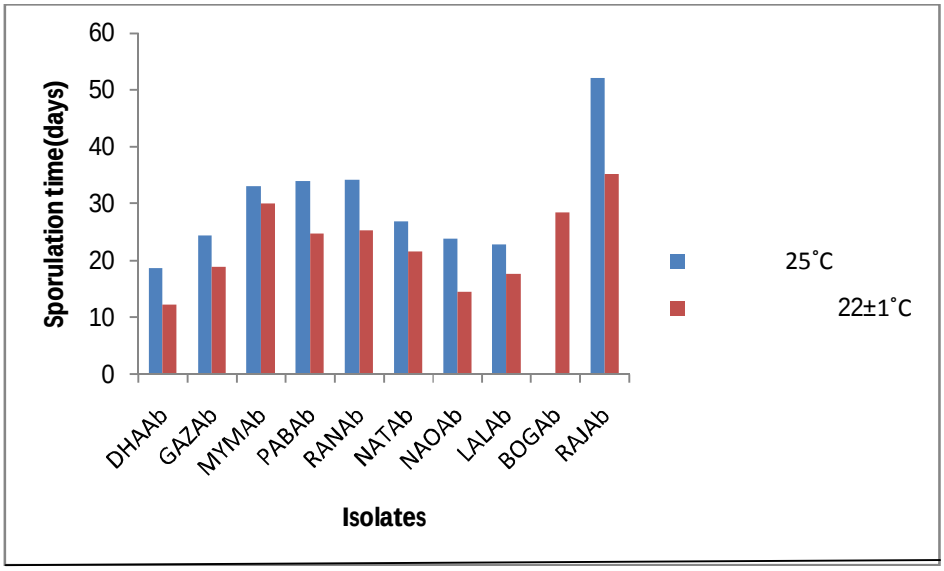


Figure 15. Effect of different temperature on sporulation time of different isolates of *Alternaria* species.

DISCUSSION

A laboratory experiment was carried out at Plant Pathology Laboratory of Oil seed Research Center, BARI, Joydevpur, Gazipur to find out effect of temperature on growth and sporulation among ten different isolates of *Alternaria* species isolated from mustard leaf having typical symptoms of Alternaria blight.

Leaves of mustard having typical symptoms were collected from ten different location of Bangladesh and causal organisms were isolated on PDA medium. All the isolates produced light brown to deep brown murifrom conidia with beak. This findings was supported by Kumar *et al*., (2014) and Giri, (2013). They were also found murifrom conidia which were brownish black. Some researcher worked with *A.brassicae* and found murifrom, obclavate conidia with brownish black. (Shaharan., *et al.,* 2016 and Chand, 2014).

All 10 isolates showed significant variations in respect of their cultural and morphological characteristics on different media. In respect of cultural characteristics, the isolates of *Alternaria* species showed variation in mycelial growth, colony color, shape, textures, subsurface color, zonation conidia production and sporulation time.

Remarkable effect of different culture media on radial mycelia growth was observed in *Alternaria* species. At 25˚C the maximum (79-90 mm) radial mycelial growth of *Alternaria* species was found on PCDA medium. The minimum (76.67-88.33 mm) radial mycelial growth was recorded on PDA medium preceded by CDA medium (83.67-90 m). The present studies showed that *Alternaria* species grows well on PCDA medium and at 25˚C. This result are partially supported by Meena, (2016), Singh *et al.,* (2015) and Tanya *et al.,* (2014) they also observed

that PCDA is suitable for mycial growth of *A. brassicae*. Khan (2011) and Yadav *et al.*, (2016) stated that 25˚C is suitable for mycial growth.

Significant variation was found in colony color of *Alternaria* species on PDA medium. Most of the colony color of the isolates were olivacious green to black. The results are partially agreement with Sofi *et al.,* (2013) who found that the colony color of *A. mali* isolated from apple was light to dark olivacious with greenish or brownish tinge. Muthukumar, (2013) found in case of *A.alternata* isolated from ribben plants colony colour black to olivaceous-black or grayish colour on PDA medium. Deep *et al.,* (2014) observed thirty two isolates of *A brassicicola* for colony color and radial growth. Colony colour of *A. brassicicola* varied from olive green to dark olivacious black on PDA.

All the isolates of *Alternaria* species colony had circular shaped. The results are in agreement with Yadav *et al.,* (2016) were identified its morphological and cultural characters of *A.brassicae* isolates from four different locations, colonies of all the isolates were circular in shape. Singh *et al.,* (2012) who found that the colony shape of *A. solani* isolated from tomato plants was circular margin with smooth surfaced colony. All the isolates colony had cottony and velvety texture on PDA medium. The results are in agreement with Barry *et al.,* (2002) examined 308 isolates of *Alternaria* spp. colonies generally had a cottony texture on group 4. Sofi *et al.,* (2013) stated Alternaria blotch, causal organism *A. mali,* colonies varied in their cultural behaviour ranging from velvety to cottony. Remarkable variations were observed on spore production and sporulation time on different media and temperature. Potato Carrot Media are found suitable for spore prodution and sporulation time for maximum isolate followed by CDA and PDA. This result are supported by the Meena, (2016) found potato carrot broth are suitable for sporulation and spore production *A. brassicae.* Sharma, (2013) found variation in mycial growth, sporulation in different nutrient media like Potato Dextrose Agar,

Cauliflower Agar medium and Carrot Potato Agar good for 32 isolates of *A. brassicae*.

Variations were observed in accordance with length, breadth and beck on different isolates of *Alternaria* species on PDA media. The length of conidia of different isolates varied from 41.56µm to 117.54µm. The breadth of conidia of different isolates varied from 10.34 µm to 23.12 µm. The beck of conidia of different isolates varied from 16.78 µm to 72.65 µm. The horizontal septation varied from 3-7 to 7-11. The vertical station varied from 0-1to 2-3. This result are partially supported with Shaharan *et al.,* (2016) define *A. brassicae* length of conidia varied from 96 µm -114 µm, bredth varied from 17 µm -24 µm and beck length varied from 45 µm-65 µm and transverse and longtidunal septation varied from 10-11 and 0-6 respectively. Kaur *et al.,* (2007) studied 322 isolates of *A. brassicae* variation was recorded among conidial length, breadth and beak length which range of 51.4-481.2 µm, 6.9-36.0 µm and 16.3 - 266.9 µm respectively. Average numbers of horizontal septa were 9.7, vertical septa were 0.8. Shakti *et al.,* (2013) stated 5 different isolates of *A.brassicae* the horizontal septation varied from 4-13 and vertical from 0-6. Saha, (2016) collected 23 isolates of *A. brassicae* and found maximum length of conidia ranged from 150 - 122 µm with 8 - 9 transverse and 2 vertical septation. Nikam *et al.,* (2015) examined eight isolates of *A. solani* and found average conidial size (L×B) were 42.18×15.18 µm and beck size were 13.10µm. Jadav *et al.,* (2011) found that in ten isolates of *A. macrospora* size of conidia ranged from 20.81-56.23 x 9.2- 27.10 µm with 1 - 6 transverse and 0 - 4 longitudinal septa. Ramjegathesh and Ebenezar (2012) collected ten isolates of *A. alternata* the length and width of conidia were varied from 30.99 -42.47 µm and 11.90-17.37 µm respectively. All isolates produced both beaked and unbeaked conidia. The beak length of conidia varied from 18.7-23.81 µm. Sofi *et al*., (2013) stated Alternaria blotch, causal organism *A. mali* 21 isolates of *A. mali* were collected from different locations. Average conidial size ranged from 21.36 to 31.74 x 8.34 to 14.48 µm. Soo-Sang *et al.,* (2016) found

among isolates of *A.mali* size of conidia 19–50 µm ×5–9 µm in nature and 20–59 µm ×8–13 µm in culture, with 3–8 transverse septa and usually no longitudinal septa or only 1 longitudinal septa.

All the isolates of *Alternaria* species produced spore from 4-11 days this result is partially supported by Moshin *et al.*, (2016) found twenty seven isolates of *A. porri* produced spore from 3-11 days.

SUMMARY AND CONCLUSION

Mustard (*Brassica* spp.) is the principal oil-producing crop of Bangladesh and Alternaria leaf blight caused by *Alternaria* species, is one of the major disease of mustard. This research was conducted to find out effect of temperature causing Alternaria leaf blight of mustard on the basis of cultural and morphological aspects. The experiment was laid out in the completely randomized design with three replications. Ten isolates of *Alternaria* species were collected from ten different mustard growing districts of Bangladesh. Three different media and two different temperature were used to measure growth and development of *Alternaria* species.

All the 10 isolates showed variation in the terms of cultural and morphological characteristics. Among three different culture media, potato carrot agar medium at 25˚C showed the best performance in the terms of radial mycelial growth where the radial myceial growth recorded 90 mm at 14 days after incubation. The lowest radial mycelial growth was observed on PDA at 22±1˚C where the radial myceial growth recorded 69.00 mm at 14 days after incubation.

Colour of the colonies of *Alternaria* species showed variation among ten isolates. Olivacious green to black color colony developed on PDA medium. All the isolates produced Circular colony and the texture were cottony to velvety. All isolates showed compact type compactness. Variation also observed between surface and sub surface colour. Surface colour varied from light brown to deep brown. Subsurface colour varied from light brown to black and pinkish. Zonation were present both surface and subsurface in some isolates and some isolates showed no zonation on both side.

Effect of media on sporulation significantly differed among the isolates. The highest number of conidia production was recorded 48.17 to 59.79 × 10^6/ml was counted RAJ_{Ab}on Potato Carrot media at 25˚C temperature. Of all the isolates of *Alternaria* species with maximum in isolate RAJ_{Ab} and minimum in NAO_{Ab}. Temperature showed an influnce on sporulation. The highest sporulation recorded in all isolates at 25˚C which was found slightly lesser at 22±1˚C temperature.

Effect of media on sporulation time differed significantly among the isolates. The minimum days (4 days) required for sporulation in PCDA followed by CDA. Minimum time required for sporulation at 25˚C compared to 22±1˚C in all isolates tested.

Remarkable variation among different *Alternaria* species isolates were observed in length, breadth and beak size of the conidia. The conidial length varied within a range of 41.56 µm to 117.54 µm and the breadth were varied from 10.34 µm to 23.12 µm . All isolates were muriform and deep brown to light brown in colour with a beak length of 16.78 µm to 72.65 µm.

On the basis of the above results and discussion it can be summarized that- Potato Carrot agar medium and 25˚C temperature were appeared to be the best medium and temperature respectively for the mycelial growth and sporulation of this fungal pathogen. More research should be conducted on molecular characterization of this isolates to find out the phylogenetic relationship.

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