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# INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITY ON THE SORULATION AND GROWTH OF THE FUNGUS (Alternaria mali), CAUSAL AGENT OF ALTERNARIA LEAF SPOT/BLOTCH OF APPLE (Malus domestica BORKH.)

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**ABSTRACT**

*Slide germination technique (SGT) described by Wellman and McCallan (1943) was used to determine the effect of temperature on the spores of fungus and the method used by McLean and Cook ( 1941 ) was used to determine the effect of relative humidity on spore germination. Spores germinated at all temperatures from 10-40 0 c but maximum germination (98.50 percent) was obtained at 30 0 c. Although 90.00 – 93.75 per cent spores germinated within a range of 25-35 º c, but there was decline in spore germination at temperatures below 20ºc and above40 ºc. Also, the spores of Alternaria mali germinated at all the test humidity levels ranging from 46.8 to 100 per cent, however, maximum spore germination (97.9 %) was observed at 100 per cent relative humidity followed by 92.0 per cent R.H (92.17 %) which were statistically at par with each other followed by 82.0 (71.31%), 75.6 (64.40), 66.8 (52.41) and 46.8 percent (18.8%) relative humidity levels. No spore germination was observed at 36.8 percent humidity.*

***Keywords: Slide germination technique, Alternaria mali, Alternaria leaf blotch, spore germination***

## I. INTRODUCTION

Red-delicious apple (*Malus domestica* Borkh.) is the most common variety grown in the Kashmir valley. It is identified as leading producer of apple in India which contributes a major portion of about 65% of total production in India, ranks 7 th with an annual production of apple fruit (FAO,2012).Like other horticultural crops apple is attacked by several pathogen which impair the quality and quantity of the fruit. Mostly, losses in huge of the crop are caused by fungal disease like collar rot, root rot, scooty blotch, powdery mildew, Alternaria leaf blotch, scab etc. Among these Alternaria leaf blotch caused by Alternaria mali is prevalent in all the apple growing regions of the world and is one of the economically important apple diseases. Now, it has become the

problem and challenge in Jammu and Kashmir to get rid of this disease in order to prevent from huge loss of crop. An outbreak of this disease in Kashmir was noted in year 1992 and the occurrence of the disease (*Alternaria mali)* was reported by Shahzad et.al in Kashmir valley of Jammu and Kashmir. Alternaria leaf spot disease caused by *Alternaria mali* is a fungus disease recognized as important pathogen of apples resembled as frog-eye leaf spot appearance .Lesions appear as circular brown spots measuring 2-5 mm in diameter and sometimes can result in significant defoliation, decrease fruit quality and marketability due to severe infection .

Through the early part of the fruit production season the pathogen stays relatively inactive, causing only small lesions and often not being observed at all. The disease develops explosively following heavy summer rainfall events and high humidity. Trees that have mite infestations are predisposed to rapid disease development. Secondary spread of the disease occurs where spores (conidia) that develop on lesions are splashed by windblown rain. This dispersal is relatively rapid, and entire orchard blocks are quickly infected (Anonymous, 2013d). Primary infection takes place about one month after petal fall. At optimum temperatures, infection occurs with 5.5 h of wetting, and lesions can appear in the orchard two days after infection, causing a serious outbreak. The fungus produces a chemical toxin which increases the severity of the disease on susceptible cultivars (Yoder and Biggs, 1998). In valley of Kashmir, the summer of 2013 reported excessive hot temperatures and in the month of July there were heavy and consistent rains which prolonged for long periods coupled consistently with high temperatures. Such conditions favored the disease and within no time due to continuing rain and favorable temperatures for a period of several weeks, the disease spread rapidly. It is unusual for the valley of Kashmir to have such a combination of high temperature and a sudden and prolonged rainfall during this period of year, however probably it might be due to the effects of climate change scenario leading to erratic rainfall behavior and rise in temperature which resulted in this minor disease causing major losses in apple in Kashmir.

The aim of this study was to estimate the disease incidence and evaluate effect of temperature and relative humidity on growth and sporulation of the fungus.

## II. MATERIALS AND METHODS

### 1. SURVEY FOR THE LEAF INCIDENCE AND DISEASE SEVERITY

A brief survey of important apple growing belts of Kashmir valley was conducted to assess the disease incidence and intensity of Alternaria leaf spot of apple. One hundred plants of orchards of different districts such as pulwama, Shopian and Budgam were randomly selected. The leaves of the plants bearing Alternaria leaf symptoms were picked up and counted to estimate the disease incidence percentage.

### 2. COLLECTION OF DISEASED PLANT MATERIAL

Symptoms of Alternaria leaf spot on apple leaves were collected and brought to the laboratory in clean polythene bags and stored in refrigerator at $4\pm 1^0$Cfor further investigations.

## 3. ISOLATION AND MAINTENANCE OF PATHOGEN

For isolation and maintenance, diseased leaves were collected from different locations. These were washed by running tap water and dried under turn papers. Small segments of the diseased tissues were made by a sharp sterilized blade along with some healthy portions. These segments were surface sterilized in 0.1% mercuric chloride (Hgcl2), for about 30 seconds followed by three washings in sterilized distilled water. Then the segments were blotted on the filter papers and transferred into plates containing potato dextrose agar (PDA) and incubated at 25 +_$1^0$ c for three days. The mycelium emerging from diseased bits was aseptically transferred to fresh PDA culture slants. These slants were incubated at 25± $1^O$ c for 7 days. For the further characteristics of isolates the procedure of Khandewal and Prasad was adopted.

## 4. IDENTIFICATION OF THE PATHOGEN

The fungus pathogen *Alternaria mali* was identified by its colony characteristics and morphological characteristics.

## 5. EFFECT OF TEMERATURE ON SPORE GERMINATION OF THE FUNGUS

Slide germination technique (SGT) described by Wellman and McCallan (1943) was used to determine the effect of temperature on the spores of fungus 100 spores per microscopic field at 10 x power of magnification. Cavity slides with spore suspension were placed in moist chambers made by placing filter papers in petriplates. These petriplates were inoculated at seven different temperatures viz.,,$10^0$, $15^0$,$20^0$, $25^0$,$30^0$, $35^0$ and 40 $^{\pm}1$ $^0$ c. Three replications for each temperature were maintained. The number of spores germinated and ungerminated were counted respectively after 24 hours of incubation. Spore was considered to be germinated when the germ tube length was $1/4^{th}$ of the spore diameter. Germination percentage was calculated using the formula:

Per cent germination = G/T x 100

Where G is the number of spores germinated, and T is the total number of spores counted (germinated and ungerminated).

## 6. EFFECT OF RELATIVE HUMIDITY SPORE GERMINATION OF THE FUNGUS

The method used by McLean and Cook (1941) was used to determine the effect of relative humidity on spore germination. As per the method seven different levels of relative humidity viz..100, 92.9, 82.9, 75.6, 66.8, 46.8 and 36.8 percent were maintained. To maintain the above relative humidity levels , solutions of 0, 15, 25, 30, 35, 45 and 50 percent sulphuric acid respectively were prepared and poured in different petriplates. One drop of the conidial suspension containing approximately 5 x 10 4 conidia/ml was placed on cavity slide. The slide was kept

in the petriplate at different humidity levels and incubated at 25 0 c. The data on percent conidial suspension was recorded after 24 hours.

### 2.2 DATA ANALYSIS

The data obtained from laboratory as well as field experiments were subjected to appropriate statistical analysis wherever necessary using standard procedure as described by Gomez and Gomez (1984).

### 2.3 RESULTS AND DISCUSSION

### 1. SYMPTOMATOLOGICAL STUDIES IN VIVO

Being a foliar disease the symptoms on apple leaves of *Malus domestica* Borkh. were recorded periodically in the field. Initially the disease spots were minute, round, light brown in colour and measured 4-5 mm in diameter. The spots latter on turned dark brown and finally turned grayish brown in color and changed from regular to irregular in shape, acquiring a "frog-eye appearance". In case of severe infections, the spots coalesced and undergo secondary enlargement to form necrotic areas. Lesions occurred on petioles turned the leaves yellow and resulting in premature defoliation. The disease spots first appeared in the middle of leaf and then progressed towards leaf margins. Characteristic concentric rings were observed initially but disappeared with the advancement of disease.

### 2. PATHOGENICITY TESTS

Artificial inoculations of isolated fungi on injured and uninjured detached leaves of *Malus domestica* Borkh. revealed the development of typical disease symptoms on injured leaves after 7 days of inoculation. However, no such symptoms developed on uninjured leaves even after 15 days of inoculation. These symptoms almost resembled the symptoms of the diseased leaves from which the isolations were made. Reisolations from these infected leaves yielded the same fungus, thus fulfilling the Koch's postulates.

### III. EPIDEMIOLOGY OF THE PATHOGEN

The epidemiological studies on *Alternaria mali*, the major pathogen involved in the Alternaria leaf spot disease of apple (*Malus domestica* Borkh.) were carried out in liquid media under invitro conditions, so as to determine the best optimum temperature and relative humidity for the growth and sporulation of the fungus.

### 3.1 EFFECT OF DIFFERENT TEMERATURE ON SPORE GERMINATION OF THE FUNGUS

The data on the effect of various temperatures on the germination of *Alternaria mali* spores is presented in table1, figure 1.The perusal of the data revealed that the spores germinated at all temperatures from 10- $40^0$ C but maximum germination (98.50 percent) was obtained at 30 $^0$ C. Although 90.00 – 93.75 per cent spores

germinated within a range of 25- 35$^0$ C, but there was decline in spore germination at temperatures below 20$^0$ C and above40 $^0$ C.

### 3.2 EFFECT OF RELATIVE HUMIDITY ON SPORE GERMINATION OF THE FUNGUS

Data presented in the table 2 figure 2 revealed that the spores of Alternaria mali germinated at all the test humidity levels ranging from 46.8 to 100 per cent, however, maximum spore germination (97.9 %) was observed at 100 per cent relative humidity followed by 92.0 per cent R.H (92.17 %) which were statistically at par with each other followed by 82.0 (71.31%), 75.6(64.40), 66.8 (52.41) and 46.8 percent (18.8%) relative humidity levels. No spore germination was observed at 36.8 percent humidity.

Table 1 EFFECT OF DIFFERENT TEMERATURE ON SPORE GERMINATION OF THE FUNGUS

| **Temperature** | **Per cent spore germination** | | | |
|---|---|---|---|---|
| | **R$^1$** | **R$^2$** | **R$^3$** | **Mean** |
| 10 | 15.25 (22.99) | 14.90 (22.71) | 17.10 (24.43) | 15.75 (23.18) |
| 15 | 48.00 (43.85) | 50.75(45.43) | 49.00(44.43) | 49.25 (44.57) |
| 20 | 75.90 (60.60) | 77.10 (61.41) | 75.30 (60.20) | 76.10 (60.74) |
| 25 | 90.25 (71.81) | 94.50 (76.44) | 96.50 (79.22) | 93.75 (75.82) |
| 30 | 99.50 (85.50) | 97.75 (81.37) | 98.25 (80.40) | 98.75 (83.24) |
| 35 | 89.50 (71.09) | 91.00 (72.54) | 89.50 (71.09) | 90.00 (71.57) |
| 40 | 53.25 (46.84) | 55.00 (47.87) | 56.00 (48.45) | 54.75 (47.72) |
| **CD (P= 0.05)** | | | | **3.18** |

*Figures in parenthesis are angular transformed values

FIG.1 EFFECT OF DIFFERENT TEMERATURE ON SPORE GERMINATION OF THE FUNGUS

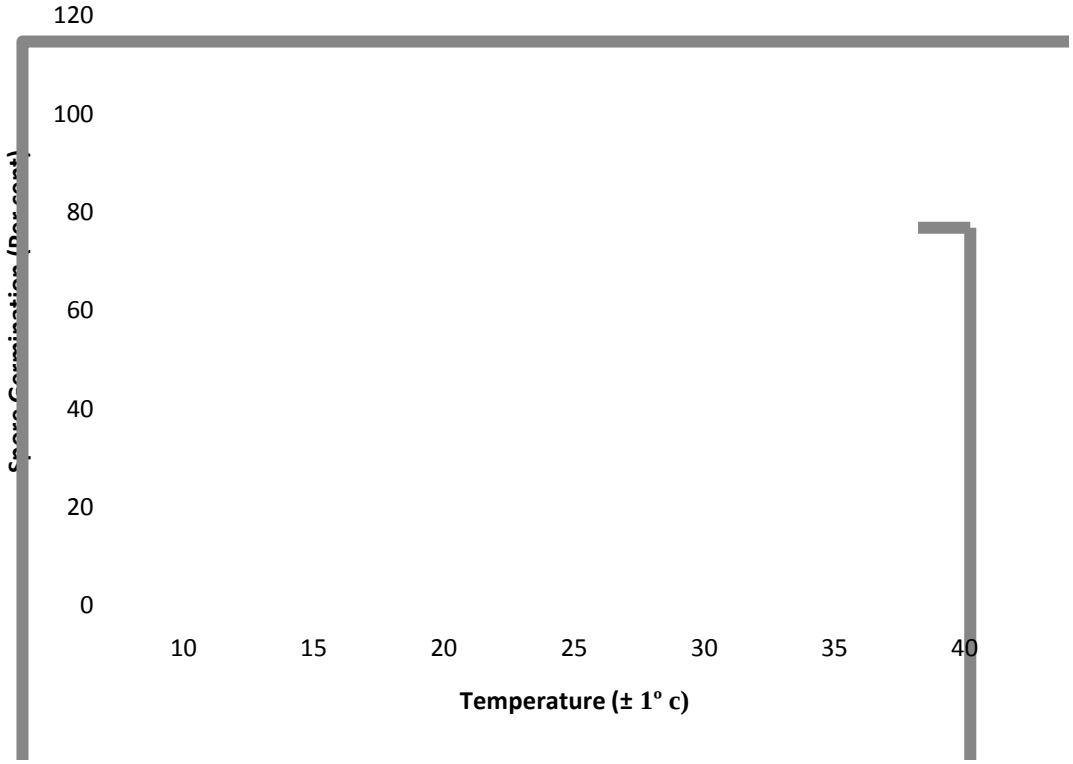


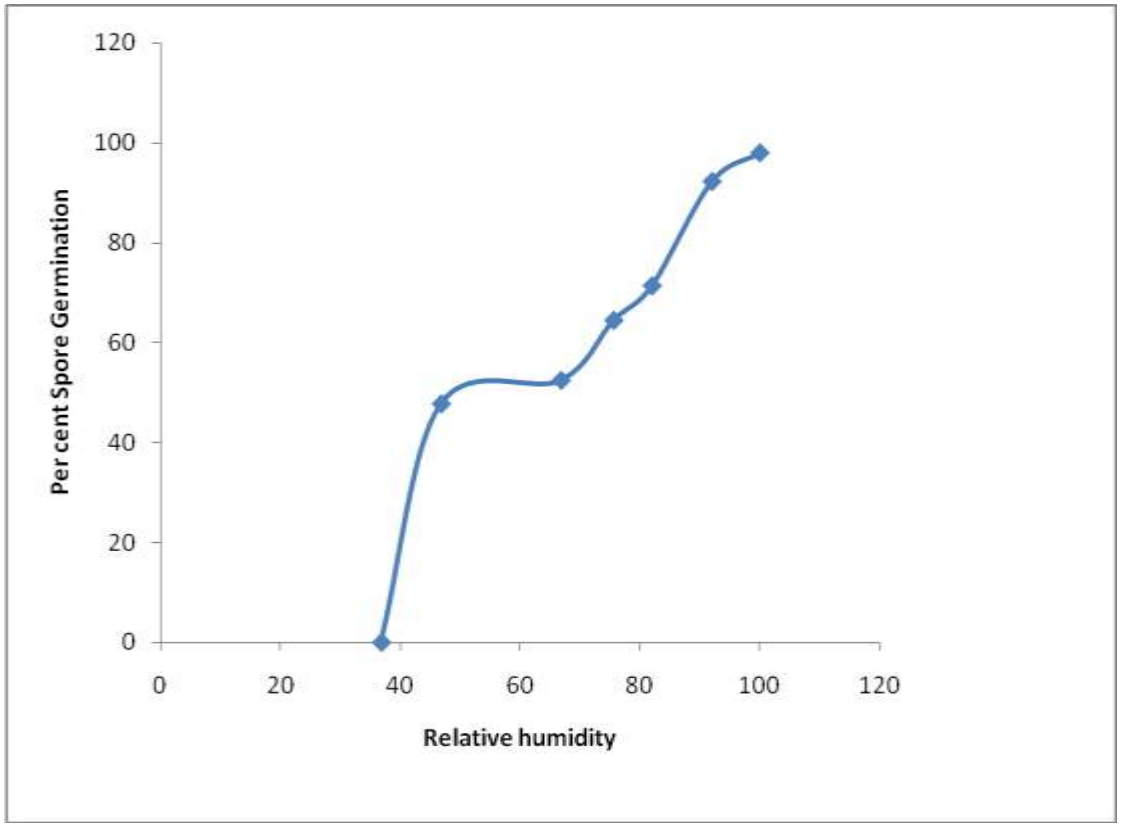

Table 2 EFFECT OF RELATIVE HUMIDITY ON SPORE GERMINATION OF THE FUNGUS

| **Relative humidity** | **Per cent spore germination** | | | |
|---|---|---|---|---|
| | **$R^1$** | **$R^2$** | **$R^3$** | **Mean** |
| 100 | 95.50 (71.81) | 98.70 (79.22) | 99.50 (76.44) | 97.9 (75.82) |
| 92.0 | 93.50 (71.09) | 92.00 (72.54) | 90.50 (71.09) | 92.17 (71.57) |
| 82.0 | 70.08 (55.00) | 72.67 (57.00) | 71.19 (53.25) | 71.31 (55.08) |
| 75.6 | 63.60 (52.73) | 64.41 (54.00) | 62.20 (55.00) | 64.40 (53.24) |
| 66.8 | 51.25 (44.25) | 52.00 (48.63) | 54.00 (50.25) | 52.41 (47.71) |
| 46.8 | 16.56 (21.25) | 18.63 (23.12) | 21.21 (25.27) | 18.8 (23.22) |

| | | | | |
|---|---|---|---|---|
| 36.8 | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |

CD (P= 0.05) 1.80(1.89)

Fig.2 EFFECT OF RELATIVE HUMIDITY ON SPORE GERMINATION OF THE FUNGUS

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## REFRENCES

[1] Anonymous, *Department of horticulture*, Stastical section, Govt.of Jammu and Kashmir,1192. [2]Shahzad A, Bhat GM "Status of Alternaria leaf blotch of apple (*Malus domestica*) in Kashmir",*SKUAST J*. Res.*vol. 7*,pp. 91- 194,2005.

[3] Filajdic.N and Sutton.T.B, "Influence of temperature and wetness duration on infection of apple leaves and virulence of different isolates of Alternaria mali", *Plant Disease,vol.82,issue 11*,1279-1283,1992.

[4] Sawamura "Data sheets on quarantine pests of Alternariamali",*Journal of EPPO quarantine pests*,1972

[5] Johnston A, Booth C, "Plant Pathologists Pocket Book", *Common Wealth Mycological Institute, Kew, Surrey, England*. p. 439,1983

[6] Saito .A, Nakazawa. N and Suzuki.M , "Selection of mutant resistant to Alternaria blotch from in vitro-cultured apple shoots irradiated with X-and gamma rays",*Journal of plant physiology,vol.158*,pp.391-400,2001.

[7] Arain .R.A,Jiskani.M.M,Wagan.H.K,Khuhro.N.S and Khaskheli.I.M (2012).Incidence andchemical control of okra leaf spot disease.*pak.j.bot.,44(5)*:1769-1774.

[8] Abe.K,Iwanami.H,Kotoda.N,Moriya.S and Takahashi.S (2009).Evaluation of apple genotypesand Malus species for resistance to Alternaria blotch caused by Alternaria alternate apple pathotype using detached-leaf method. *Plant breeding* .,129:208-218.

[9] Anderson.B,Kroger.E and Roberts.G (2001).Chemical and morphological segregation ofAlternariaalternate,A.gaisen and A.longipes.*Mycol.Res.,105(3)*:291-299. [10]Bhat.A.K,Peerazada.H.S and Anwar.A (2015).Alternaria epidemic of apple in kashmir.African *Journal Of Microbiology Research.,9(12)*:831-837.

[11] Bhat.A.H,khurshid.A,Ahanger.A.R,Qazi.A.N,Dar.A.N and Ganie.A.S (2013).Status and symptomatology of Alternaria leaf blight(Alternaria alternate) of Gerbera(Gerbera jamisonii) in Kashmir valley.*African Journal of Agricultural Research.,8:819-823*.

[12] Chandrashekar. M, and Ball.C.M(1980)..Leaf blight of grey mangrove in australia caused by AlternariaAlternata .*Journal of Trans ,Br,mycol,Soc.,75(3)*413-418.

[13] Colson-Hanks.S.E and Deverall.J.B(2000).Effect of 2,6-dichloroisonicotinic acid ,its formulation materials and benzothiadiazole on systematic resistance to alternaria leaf spot in cotton.*Plant Pathology.,49*:171-178.

[14] Dickinson.H.S and Wallace.B (1976).Effects of late applications of foliar fungicides on activity of micro-organisms on winter wheat flag leaves.*Trans.mycol.Soc.76(1):*103-112.

[15] Dickinson.H.S and Bottomley.D (1980).Germination and growth of Alternaria and Cladosporium in relation to their activity in the phylloplane.*Trans.Br.mycol.Soc.74(2)*:309-319.

[16] Filajdic.N and Sutton(1995).Overwintering of Alternariamali,the causal agent of Alternariablotch of apple.*Plant Disease.79*:695-698.

[17] Filajdic.N,Sutton.T.B,walgenbach.J.F and Unrath.C.R(1995).The influence of apple aphidaphid complex on intensity of Alternaria blotch of apple and fruit quality characteristics and yield. *Plant Disease.,79*:691-694.

[18] Filajdic.N,Sutton.T.B,Walgenbach.J.F and Unrath.C.R(1995).The influence of European red mites on intensity of Alternaria blotch of apple and fruit quality and yield.*Plant Disease.,79:*683-690.

[19] Filajdic.N and Sutton.T.B(1992).Chemical control of Alternaria blotch of apples caused by Alternariamali.*Plant Disease.,76*:1126- 130.

[20] Filajdic.N and Sutton.T.B(1992).Influence of temperature and wetness duration on infection of apple leaves and virulence of different isolates of Alternariamali.*Plant Disease.,82(*11):1279-1283.

[21] Guleria.S and Kumar.A(2006).Azadirachtaindica leaf extract induces resistance in sesame against Alternaria leaf spot disease.*Journal of cell and molecular biology.,5*:81-86.

[22] Hong.X.C,Fitt.L.D.B and Welham.J.S(1996).Effects of wetness period and temperature on development of dark pod spot (Alternariabrassicae) on oilseed rape (Brassica napus).*Plantpathology.,45*:1077-1089.

[23] Hutton.D.G(1988).The appearance of dicarboximide resistance in Alternaria alternate in passionfruit in south-east Queensland.Australasian *Plant Pathology.,17*:34-36.

[24] Hutton.D.G and Mayers.P.E(1988).Brown spot of murcot tangor caused by Alternaria alternate in Queensland .Australasian *Plant Pathology.,17:*69-73..

[25] Johnston A, Booth C (1983). *Plant Pathologist,s Pocket Book*. Common-wealth Mycological Institute Kew, Surrey England.

[26] Jung.H.K(2007).Growth inhibition effect of pyroligneous acid on pathogenic fungus,Alternaria mali,thecagent of Alternaria blotch of apple.*Biotechnology and bioprocess engineering.,12:*318-322.

[27] Khandare.K.N(2014). Efficacy of Carbendazim and other Fungicides on the Development of Resistance during Passage in AlternariaAlternata Causing Root Rot to Fenugreek. *International journal of science and research.,3(10):*2115-2119.

[28] Kohmoto.K,Khan.D.I,Renbutsu.Y,Taniguchi.T and Nishimura.S(1976).*Physiological Plant Pathology.,8*:141-153.

[29] Kumar A (2004). Cultural and physiological variability among the isolates of Alternariatriticina from Bihar causing leaf blight of leaf. *Plant Dis. Res. 19*:10-15.

[30]Logrieco.A,Moretti and Solfrizzo.M(2009).Alternaria toxins and palntdiseases:an overview of origin,occurrence and risks.*WorldMycotoxin Journal.,2(2*):129-140.

[31] Mamgain.A,Roychowghury.R and Tah.J(2013).Alternaria pathogenicity and its strategic controls.Research *Journal of Biology.,1*:01-09.

[32] Mathivanan.N and Prabavathy.R.V(2007).Effect of carbendazim and mancozeb combination Alternaria leaf blight and seed yield in sunflower(Helianthus annus L.).*Archives of phytopathology anf plant protection.,40(2*):90-96.

[33] Odonnelland Dickinson.H.C(1980).Pathogenicity of Alternaria and Cladosporium isolate on phaseolus.*Trans.Br.mycol.Soc.,74(2);*335- 342..

[34] Singh.N.S(2002).Effect of sowing dates and fungicidal spray onAlternaria blight and yield of sunflower .*Journal of Indian phytopath.,55(1):*104-106.

[35] Sofi.T.A,Beigh.A.M,Dar.H.G,Ahanger.A.F,andHamid.A.(2013).Virulence variation in Alternariamali (Roberts)and evaluation of systematic acquired resistance(SAR)activators for the management of Alternaria leaf blotch of apple. *Emirate journal of food and agriculture ,.25(3):*196-2014.

[36] Sofi.A.T,Muzafer.A.B,Dar.H.G,Mushtaq.A,Hamid.A,Ahanger.A.F,Padder.A.and Shah.D.M (2013).Cultural ,morphological ,pathogenic and molecular characterization of Alternariamali associated with Alternaria leaf blotch of apple. *African journal of biotechnology .,12(4)* : 370- 381 .

[37] Soliemani.J.M,and Esmailzadeh.M,(2002).First report of Alternariamali causing apple lea blotch disease in iran. *Australasian Plant Disease.,2:57-58.*

[38] Yoder KS, Biggs AR (1998). Alternaria Blotch. Extention Note. Extention service. *West Virgiana University Dated 11/051998.*