STUDIES ON THE LEAF BLIGHT OF ONION CAUSED BY *Alternaria cepulicola*, Rao, IN MAHARASHTRA STATE

A Thesis submitted to the

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(Agricultural University)

Rahuri, Dist. Ahmednagar (Maharashtra)

in partial fulfilment of the requirements for the degree of

Master of Science (Agriculture)

in

Plant Pathology

By

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C E R T I F I C A T E

This is to certify that the thesis entitled "Studies on the leaf blight of onion caused by Alternaria cepulicola Rao, in Maharashtra State" submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth (Agricultural University), Rahuri, District Ahmednagar (Maharashtra) in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY, embodies the results of a piece of bonafide research work carried out by SHRI PATIL ASHOK OMKAR under my guidance and supervision and that no part of the thesis has been submitted for any other degree or publication.

College of Agriculture,
Poona-5, Maharashtra.
Dated : September, 1971.

(Laiqua Ahmed)
Research Guide .

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Table of Contents

STUDIES ON
[illegible] LEAF BLIGHT OF ONION

INTRODUCTION

Onion (*Allium cepa* L.) locally known as *Kanda* is an important vegetable crop. It comes under the genus *Allium* and there are 300 species. Onion is believed to be of Asian origin, from Palestine to India. It is cultivated in America from 1629. In India onion is cultivated from many years. It is cultivated mainly in Maharashtra, Tamilnadu, Bihar, Mysore, Andhra - Pradesh, Madhya Pradesh and Gujarat.

Maharashtra occupies 89,472 acres under onion (1968-69). The distribution of area in Maharashtra State is given below :

Sr.No.	Name of the Division		Area in acres.
1.	Bombay	....	43,187
2.	Poona	....	30,948
3.	Aurangabad	....	8,805
4.	Nagpur	....	7,432
		Total :	89,472

It is a good source of vitamins 'B' and 'C'.

Composition

	Water	Fat	Proteins	Starch	Fibre	Salts.
Red Onion.	86.50	0.35	2.12	9.75	0.65	0.63
White Onion.	85.60	0.20	2.19	10.12	0.75	0.72

From India it is exported to Ceylon, Malaya, Singapore, Zanzibar, Kenya and West Pakistan.

Number of diseases have been recorded on this crop in Maharashtra. Leaf-spots incited by Alternaria spp., downy mildew by Peronospora destructor Berkeley, Neck rot by Botrytis allii Munn, Black mold by Aspergillus niger van Tieghem, damping off by Pythium spp.

Recently, while undertaking survey of the diseases on onion, a rather serious disease inciting leaf spot was noticed during rabi around Poona. Microscopic examinations and isolations made from the affected leaves of onion had always yielded Alternaria sp.

Ayyangar (1926) reported Alternaria palandui on onion in India for the first time from Coimbatore (Tamilnadu). Joshi and Agnihotri (1963) reported A. alli for the first time on onion at the Government College Ajmer at Nekri. Vasan Rao (1963) reported A. consulicola on onion for the first time in India from Poona.

Alternaria leaf-spot is becoming rather serious and causes reduction in yield to the extent of about 5 per cent. As the disease is new to Maharashtra, it was thought worthwhile to study it in detail regarding the identity of pathogen, to test some of the modern fungicides *in vivo* and *in vitro* from control point of view.

REVIEW OF LITERATURE

Nees (1816) described the genus Alternaria to include fungi having obclavate, coloured, muriform spores borne in chains as noticed in Alternaria tenuis. Fries (1825) did not recognise the genus Alternaria but included it under Torula without mentioning the genus as such. Chevallier (1826) however, recognised the genus Alternaria and gave its differentiating characters from Torula, as having 'links' of spores separated by filiform connections. Corda (1839-40) confirmed the validity of the genus Alternaria. Subsequently, Fries (1849) in his "Summa vegetabilium scandinavica" recognised the genus Alternaria and distinguished it from Macrosporium. Elliott (1917) studied the taxonomy, and limitations of Alternaria, stressing the generic characters of conidia as obclavate, pointed, often with long beak and thought that in certain cases, the chain formation could be suppressed. He considered Alternaria and Macrosporium as two distinct genera on the basis of natural morphology, cultural characters, changes in the media and host relations. Later Angell considered Alternaria and Macrosporium as synonyms and proposed the term Macrosporium to designate both the genera. Wiltshire (1933) recognised Alternaria with original concept and avoided confusion between Alternaria and Macrosporium. He suggested to place Macrosporium among "nomina ambigua" and stated that in the original description, Macrosporium was used to designate fungi

belonging to Alternaria with non-filiform spores and as such preferred the name Alternaria for designating both the genera. Thus, the validity of the genus Alternaria has now been acknowledged and the genus Macrosporium made as a synonym.

In India, Ayyangar (1928) was the first to report Alternaria on onion. He studied the disease and identified the pathogen as A. palandui n. sp. on basis of spore dimensions, cultural differences and morphological characters. Thirumalachar and Mishra (1953) reported A. porri from Bihar (India) on onion. Joshi and Agnihotri (1960) had reported a new spot on onion caused by A. allii, a new record from India. Vasant Rao (1963) reported A. tenuissima for the first time in India. He identified the pathogen on basis of morphological characters, dimensions of conidiophores and conidia and host relationship.

From the above review, it appears that except Ayyangar (1928), Thirumalachar and Mishra (1963), Vasant Rao (1963) no much work on Alternaria from onion has been done in India.

SYMPTOMS OF THE DISEASE.

In nature the disease manifested itself on leaves only in the form of dark-purplish brown, oval to irregular, scattered spots with a paler outer zone, resulting in blight and withertip.

On artificial inoculation the symptoms appeared 8 days after inoculation by the manifestation of small specks which developed into oval to irregular spots and blight in about 12 days. Drying of the leaves starts from the tip.

No other part of the plant was found affected by the disease.

Similar symptoms could also be incited on leaves alone, when the plants were artificially inoculated (Plate I).

PLATE I

Diseased leaves showing symptoms

H = Healthy leaves

D = Diseased leaves.

PATHOGENICITY OF THE FUNGUS

a) Isolation:-

Onion leaves showing typical symptoms of the disease were collected, washed with tap water, to remove dirt etc. After drying in air, small fragments were cut from diseased portions. These cut pieces were disinfected with 1: 1000 mercuric chloride solution for one minute and rinsed in three changes of sterilised water in order to remove traces of the disinfectant. These fragments were then planted in petridishes poured with potato dextrose agar, which was previously sterilised at 15 lbs. pressure for 15 minutes. The plates were then inverted and incubated at room temperatures (25° to 28° C.) for one day and then reinverted. Well developed colonies were transferred to slants of the Coon's medium and maintained for further studies.

b) Inoculation:-

Young growing cultures of the organism were used for proving the pathogenicity. The inoculations were made on local variety of onion raised in 9" earthen pots filled with sterilised soil. Two months old healthy plants were selected for inoculation. These plants were kept in moist chamber for 24 hours

before and after inoculation. Culture suspension was prepared in sterilized water with abundant spores and the same was atomized on leaves of onion with a hand atomizer. Sufficient uninoculated controls were provided which were sprayed with only sterilized water. All inoculated plants and control were removed from the moist chamber and placed in the open on glass house benches and kept under observations. The first symptoms appeared after 8 days of inoculation as small specks which developed into oval spots and blight in about 12 days while control plants were healthy. The symptoms produced in artificial inoculation were identical to those observed in natural infection.

e) Reisolation:-

Reisolations were made from artificially infected leaves which yielded a fungus which was identical in all respects to the original culture used for inoculation (Plate II).

PLATE II

Pathogenicity of the fungus

H = Healthy plants

D = Diseased plants

Host Range

Host range studies were undertaken to find out the ability of the fungus to infect other plants besides the own host. For this purpose, one month old plants which are to be tested were raised in 9" earthenware pots filled with the sterilised soil and were inoculated by spraying the spores and mycelial suspension of the fungus culture. The plants were kept in moist chambers 24 hours before and after inoculation. Adequate controls were also provided. The observations are given in Table 1 .

Table 1

Host range of *Alternaria* sp. from onion.

Sr. No.	Host Plant tested	Infection
1.	Bhendi (*Abelmoschus esculanta*, L)	+
2.	Cotton (*Gossypium arboreum*, L.)	-
3.	Chillies (*Capsicum annum*, L.)	-
4.	Coriander (*Coriandrum sativum*, L.)	-
5.	Groundnut (*Arachis hypogea*, L.)	-
6.	Jowar (*Sorghum vulgare*, L.)	-
7.	Radish (*Raphanus sativus*, L.)	-
8.	Baira (*Pennisetum typhoidas* B.& S.)	-
9.	Methi (*Trigonella foenum graecum*, L.)	-
10.	Maize (*Zea mays*, L.)	-

Table 1 (Contd.)

Sr. No.	Host Plant tested	Infection
11.	Tobacco (Nicotiana tabacum, L.)	-
12.	Matki (Phaseolus aconitifolius Jacqain).	-
13.	Soya-bean (Glycine max, (L.) Merr.)	-
14.	Gram (Cicer arietinum, L.)	-
15.	Peas (Pisum sativum, L.)	-
16.	Sann hemp (Crotalaria juncea, L.)	-
17.	Potato (Solanum tuberosum, L.)	-
18.	Urid (Phaseolus mungo, L.)	-

Note : + = Positive infection.

\- = negative infection.

The results given in the Table 1, indicate that it could incite infection on bhendi besides onion.

Varietal resistance.

This experiment was undertaken to study comparative resistance of different varieties of onion against the pathogen, and also to determine the possibility of locating the source of resistance, if any.

Plants of available varieties of onion were raised in 9" pots. When they were two and half months old they were inoculated with the spore and mycelial suspension of the fungus with atomizer. The plants were kept 24 hours prior to and after inoculation in the moist chambers. Adequate control was also provided.

The observations on the performance of these varieties recorded 15 days after inoculation are given in the Table 2.

Table 2.

Relative performance of onion varieties against *Alternaria* sp.

Sr.No.	Variety	Infection.
1.	N-53	+++
2.	N-2-4-1	++
3.	N-257-9-1	++

Rating :

+++ = Highly susceptible.

++ = Moderately susceptible.

From Table 2, it appears that none of the tested varieties of onion was resistant. The varieties N-2-4-1 and N-257-3-1 were moderately susceptible.

FUNGUS MORPHOLOGY.

The following morphological characters of the fungus were studied from the culture grown on Coon's agar and also from the affected host.

1) Mycelium :

Mycelium was septate, irregularly branched, hyaline when young and turning brown when old. The width of the mycelium from the host was 4.36 µ (2.88 to 6.24 µ) and from Coon's agar was 3.02 µ (1.92 to 4.08 µ) (Plate III, Fig. 1).

2) Conidiophores:

Conidiophores emerge by bursting the diseased host tissue, buldged at the base, short, usually straight or slightly curved, dark-olevaceous brown and were septate (2-6) . The length of conidiophores was 42.15 µ (21.84 to 98.88 µ) and breadth was 7.44 µ (6.12 to 9.76 µ) (Plate III Fig. 5).

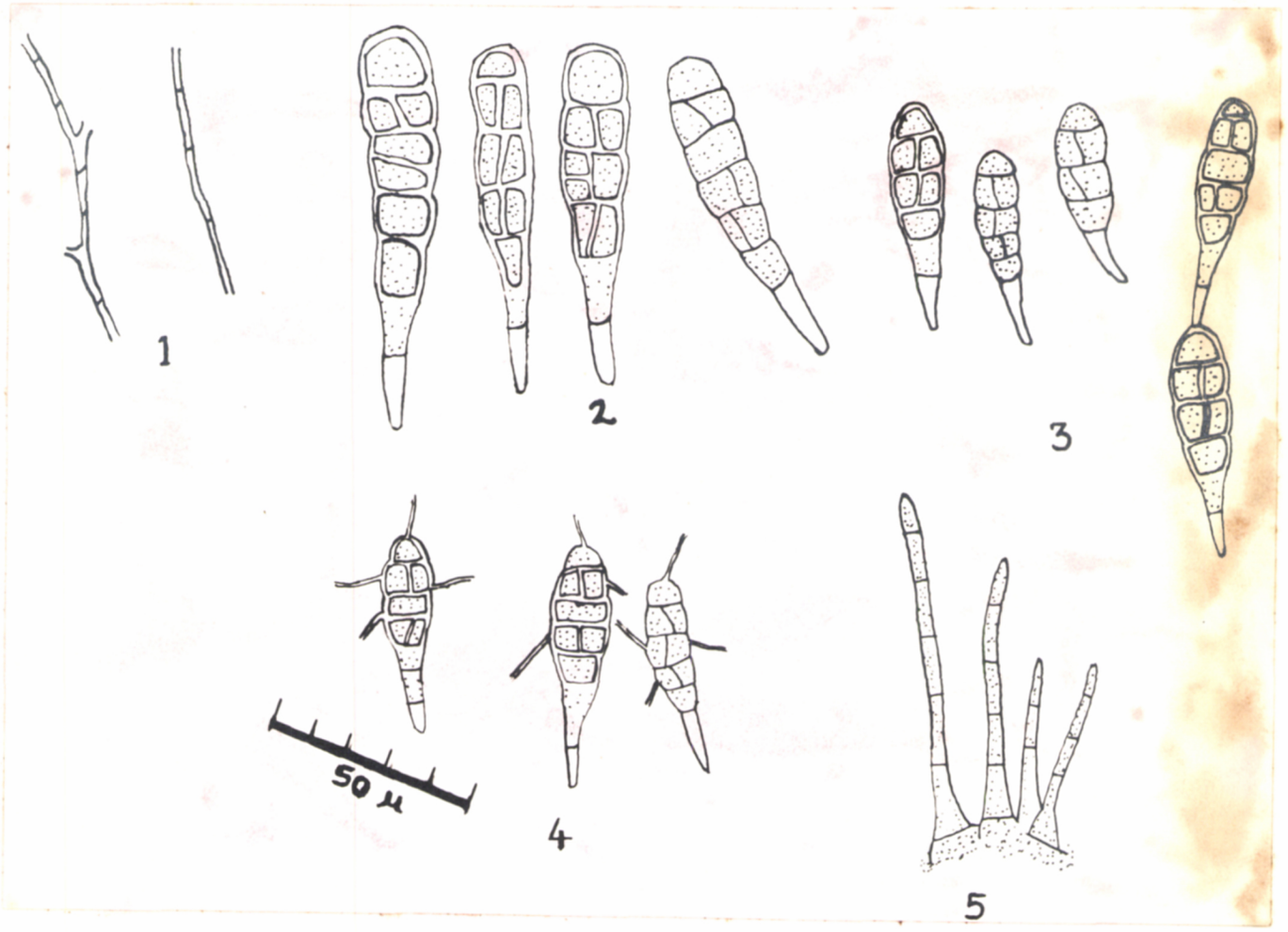
3) Conidia :

Conidia were rarely in short chains of 2 to 3 and were smaller in size from Coon's agar than those from the host. Conidia were olevaceous palebrown, mostly obclavate to muriform, mostly double-walled, the apical portion tapering to a simple beak of varying length, 2-6 longitudinal septa and 3-9 transverse septa.

They measured 68.34 μ (45.60 to 110.08 μ) X 20.14 μ (10.44 to 29.84 μ) from the host and 40.17 μ (31.28 to 59.76 μ) X 9.98 μ (6.24 to 15.84 μ) from Coon's agar (Plate III, Fig. 2 and 3).

Conidia obtained from the host had longer beaks.

PLATE III



(1) Mycelium (2) Conidia on host tissue.

(3) Conidia on Coon's agar. (4) Germination of conidia.

(5) Conidiophores in group.

SPORE GERMINATION.

Spore germination studies were undertaken to note the mode, time required and effect of different substrata on the germination of conidia of the fungus. For this purpose, the following experiment was under-taken.

Effect of different substrates.

This experiment was carried out to determine the best substratum which would induce effective germination of spores of the fungus. Series of hanging drop cultures were prepared with spores placed in different substrata and mounted over cavity slides. These slides were then kept in sterilised petri - dishes containing moist cotton wool to prevent drying and also to provide sufficient moisture necessary for germination of the spores. The plates were incubated at room temperatures (28° - 38° C). Germination started after 2½ hours and almost completed after 8 hours. Two to seven germ tubes were given out laterally and terminally. Observations on per cent germination recorded after eight hours are given in the Table 3.

Table 3.

Germination of conidia on different substrata.

Sr. No.	Substratum		Per cent germination after eight hours.
1.	Coon's medium	....	88
2.	1 % sugar solution	....	85
3.	Distilled water	....	80
4.	Sterilized water	....	82
5.	Host leaf extract	....	89
6.	Tap water	....	72

It will be seen from the results given in the Table 3, that the best substratum which induced maximum germination of spores was host leaf extract followed by Coon's medium, 1% sugar solution, sterilized water, distilled water and tap water.

Cultural characters of the fungus.

In order to study the cultural characters of the fungus, the below given solid media were used.

1) Potato dextrose agar.
2) Richards' agar.
3) M_2 agar.
4) Czapeck's agar.
5) Leonian's agar.
6) Coon's agar.
7) Nutrient agar.
8) Proteose Peptone agar.
9) Host leaf extract agar.
10) Oat meal agar.

The above given media were prepared and steri-lized at 15 lbs.pressure for 15 minutes. Each of the media were poured in the duplicate plates, and a uniform bit of the fungus secured by means of a sterilized cork borer was inoculated in the centre and incubated at room temperatures (26° - 28° C.) for 10 days. Growth characters and sporulation were studied and observations on the same were recorded. Ridgway's(1912) colour standards and nomenclature were followed for describing the colours of colonies and substratum.

The results are given in Table 4 (Plate IV).

Table 4.

Cultural characters of the fungus on different media.

Sr.No.	Name of the media used.	Mean colony dia.in mm. after 10 days.	Sporulation.	Growth Characters.
1.	Potato Dextrose agar.	76	++	Colony circular with entire margin, micelium compact with poor aerial hyphae, colony dusky neutral gray and substratum blackish plumbeous.
2.	Richards' agar.	74	+	Colony circular, undulated margin, mycelium thick, compact with poor aerial hyphae, colony quaker drab and substratum dark purple drab.
3.	K_2 agar.	88	++++	Colony Circular with entire margin, mycelium thick, compact and no aerial hyphae, colony blakish slate and substratum purplish gray.

Table 4 (contd).

Sr. No.	Name of the media used.	Mean colony dia.in mm. after 10 days.	Sporulation.	Growth Characters.
4.	Czapeck's agar.	75	+++	Colony irregular, undulated margin, mycelium thick,compact and no aerial hyphae, colony slightly raised in the centre,five concentric rings formed in the colony, colony olivaceous black and substratum haematite red.
5.	Leonian's agar.	80	++++	Colony circular with entire margin,mycelium thin and spreading with poor aerial hyphae, colony light olive gray and substratum deep olive gray.
6.	Coon's agar.	76	++++	Colony circular with entire margin,mycelium slightly thin without aerial hyphae,colony olivaceous black and substratum court gray.

Table 4 (contd).

Nr. No.	Name of the media used.	Mean colony dia.in mm.after 10 days.	Sporula-tion.	Growth Characters.
7.	Nutrient agar.	39	+	Colony circular with entire margin,mycelium slightly thin with aerial hyphae,colony deep mouse gray and substratum blakish brown.
8.	Protease peptone agar.	71	+++	Colony circular with entire margin,mycelium thick with poor aerial hyphae, colony blakish mouse gray and substratum aline black.
9.	Host leaf extract agar.	89	++++	Colony circular with entire margin,mycelium thick with good aerial hyphae,colony bluish slate black and substratum dark blue.
10.	Oat meal agar.	82	+	Colony circular with entire margin,mycelium very thick with poor aerial hyphae, colony olivaceous black and substratum deep brown-ish drab.

Sporulation:

++++ = Abundant ;

+++ = Good;

++ = Moderate;

+ = Scanty

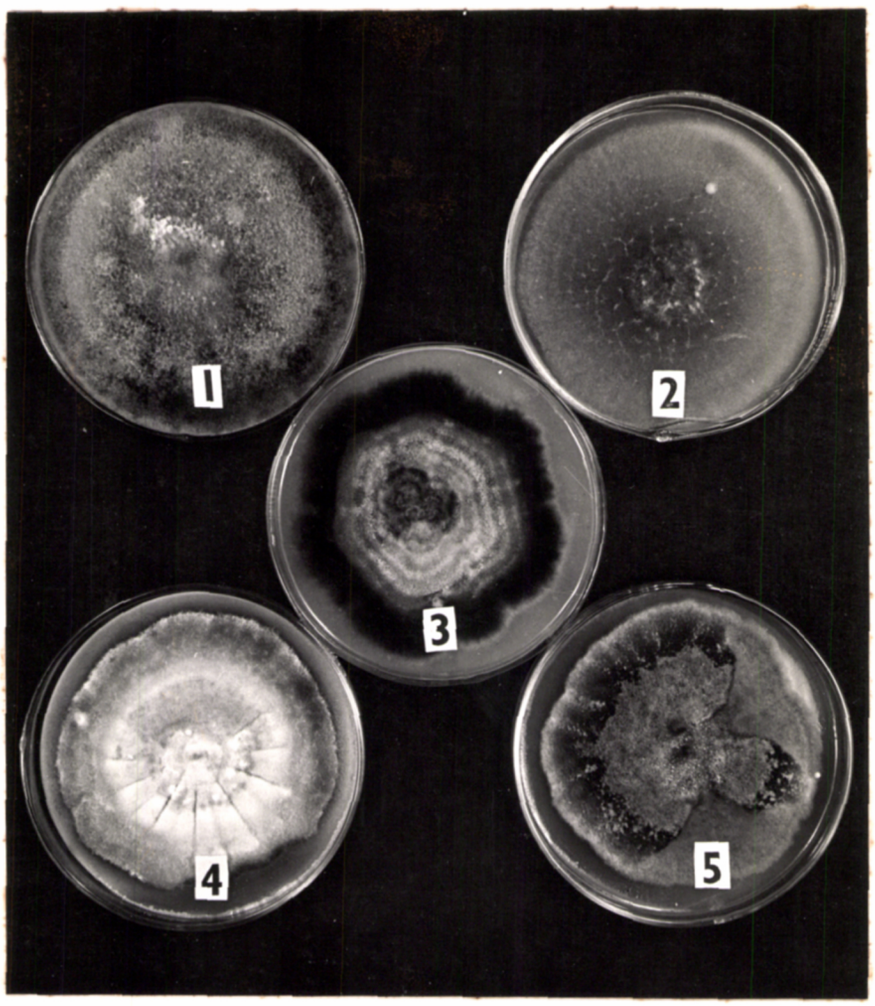
- = Nil.

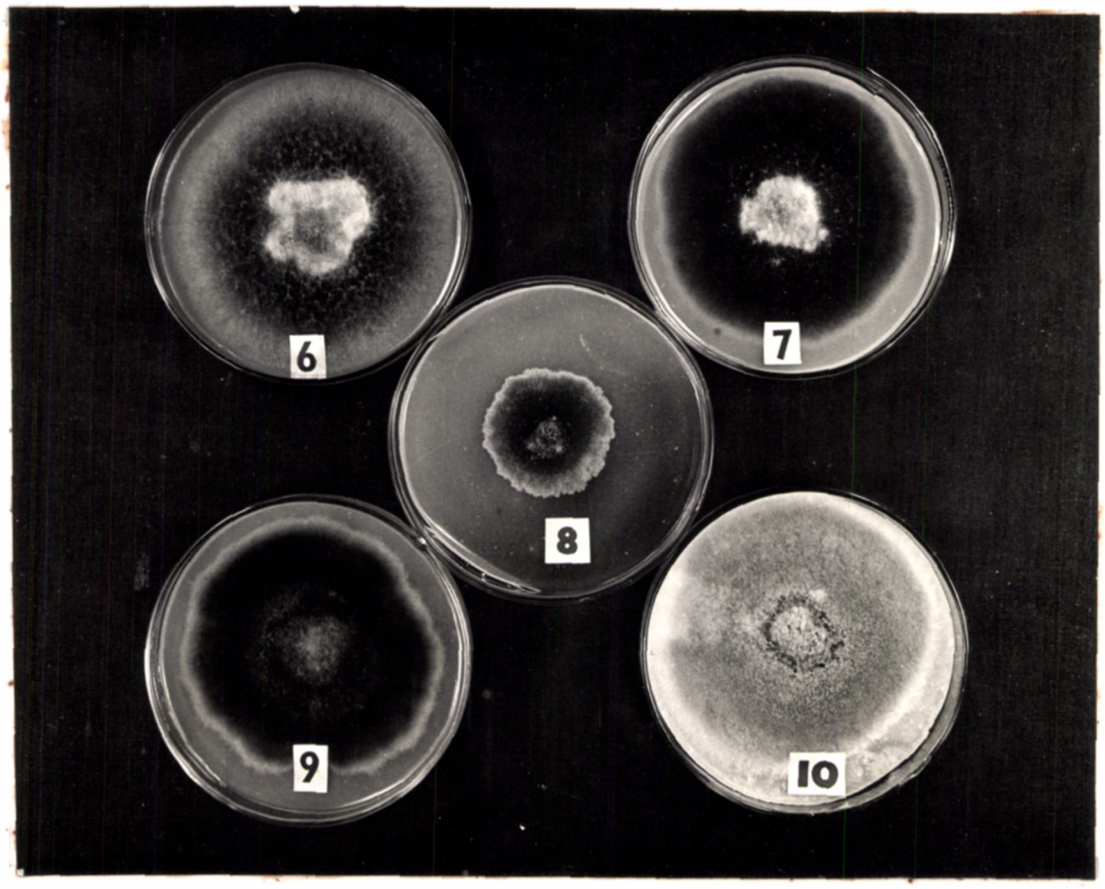
From the results shown in the Table 4, it is observed that the fungus could grow on different media viz. Potato dextrose agar, Richards' agar, M_2 agar, Czapeck's agar, Leonian's agar, Coon's agar, Nutrient agar, Proteose-Peptone agar, Host leaf extract agar, and Oat meal agar. On M_2 agar, Leonian's agar, Host leaf extract agar, Oat meal agar, the organism grew best, on potato dextrose agar, Richards' agar, Czapeck's agar, Coon's agar, Proteose Peptone agar the growth of the organism was good. On nutrient agar growth fair. On M_2 agar, Leonian's agar, Coon's agar, Host leaf extract agar, abundant sporulation was obtained, whereas good sporulation was obtained on Czapeck's agar, and Proteose peptone agar. On Potato dextrose agar sporulation was moderate. There was scanty sporulation on Richards' agar, Nutrient agar and Oat meal agar. Thus, the fungus derives nutrition from a very wide range both synthetic and non-synthetic media with the production of spores in all.

Plate IV

1) M_2 agar
2) Leonian's agar.
3) Czapeck's agar.
4) Richards' agar.
5) Potato dextrose agar.
6) Host leaf extract agar.
7) Coon's agar.
8) Nutrient agar.
9) Protease peptone agar.
10) Oat meal agar.

PLATE IV





PHYSIOLOGICAL CHARACTERS.

The following physiological characters of the fungus were studied with the object of observing the optimum temperature, pH, carbon and nitrogen requirements as well as to know the ability of the fungus to produce various enzymes, to know under what conditions the fungus could grow and survive in nature and to know thermal death point of the fungus.

The following physiological characters were studied to achieve the above purpose.

A : Temperature growth relations.

B : Utilisation of carbon compounds.

C : Utilisation of nitrogenous compounds.

D : Enzyme production.

E : Growth of the fungus in relation to hydrogen-ion concentration.

F : Thermal-death point.

A) TEMPERATURE GROWTH RELATIONS.

The relation of the temperature to the growth of the fungus was studied on Coon's agar in duplicate petriplates. The plates were poured with an equal quantity of sterilized medium. After gelling of the medium the petriplates were inoculated in the centre with equal bits of fungus from a young growing culture obtained by cork borer and incubated at various temperatures.

Observations were recorded regarding colony diameter and sporulation 8 days after incubation. The results are given in Table 5 (Plate V).

Table 5

Temperature growth relationship of the fungus under study.

Sr. No.	Temperature in O C	Mean colony dia. in mm. after 8 days.	Sporulation
1.	0	-	-
2.	5	-	-
3.	10	8	-
4.	15	18	+
5.	20	23	+++
6.	Room Temp. (25 - 28 °) .	60	++++
7.	30	55	++++
8.	35	-	-
9.	40	-	-

Sporulation:

++++ = Abundant;

+++ = Good ;

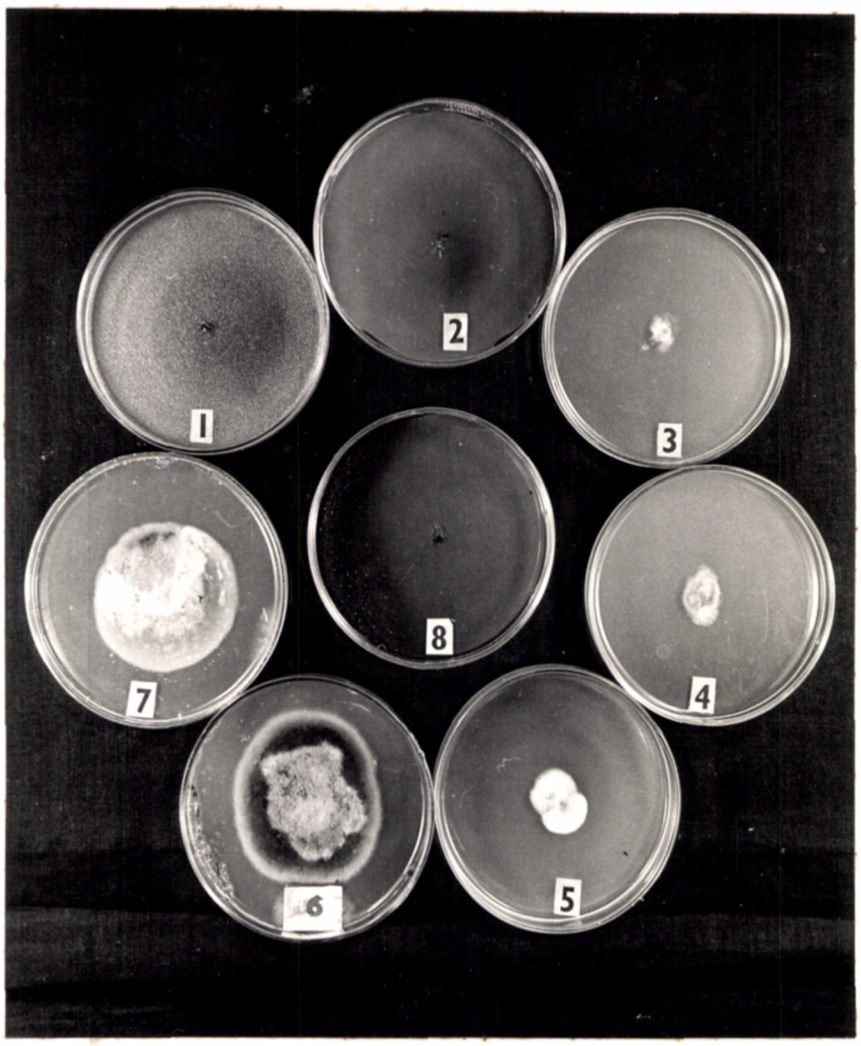
++ = Moderate ;

+ = Scanty ;

- = Nil.

It is seen from the above Table that fungus had a different range of temperature for growth , minimum temperature 10° C, and maximum of 30°C, the optimum temperature being 25° to 28° C with abundant sporulation. The fungus did not grow at 0°C ; 5°C, and 35°C.

PLATE V.



1) 0°C. 2) 5°C. 3) 10°C. 4) 15°C.

5) 20°C. 6) Room temperature. (25-28°C) 7) 30°C.

8) 35°C.

B) Utilization of carbon compounds.

In this experiment, the fungus under study was observed for its ability to use various carbon compounds, particularly carbohydrates by using a basal medium (Coon's medium without maltose) to which adequate quality of carbon compounds, calculated on the basis of carbon contents equal to those of maltose in Coon's medium were added. The medium was prepared, sterilized as usual, poured in plates, inoculated with a bit of young growing culture and incubated at room temperatures (26^{0} -28^{0} C.) The observations regarding colony diameter, sporulation and growth characters were taken on 8th day after incubation. The results are presented in Table 6. (Plate VI).

The following carbon compounds were used for the study.

1. Monosaccharides

   A. Pentoses.

      1. Rhamnose.
      2. D - xylose.
      3. Arabinose.

   B. Hexoses.

      4. Galactose.
      5. Glucose.
      6. Laevulose.

II. Disaccharides.

7. Lactose.

8. Sucrose.

III. Polysaccharides.

9. Dextrin.

10. Inulin.

11. Control (Coon's medium without maltose).

Table 6.

Sr. No.	Source of Carbon.	Colony dia. in mm. after 8 days.	Sporulation.	Growth characters.
	I. Monosaccharides.			
	A. Pentoses.			
1.	Rhamnose.	52	++	Colony regular with entire margin, mycelium well developed, massive, whitish at the centre and dirty at the peripheri.
2.	D-xylose.	48	+	Colony regular with entire margin, mycelium well developed, massive and raised at the centre, colony olivaceous black.

Table 6 (Contd).

Sr. No.	Source of Carbon.	Colony dia. in mm. after 8 days.	Sporu-lation.	Growth characters.
3.	Arabinose	51	+	Colony regular with entire margin, mycelium well developed, massive and raised at the centre, colony dirty white.
	B. Hexoses.			
4.	Galactose.	49	++++	Colony irregular with undulated margin, mycelium well developed, raised at the centre, colony olivaceous black.
5.	Glucose	44	++++	Colony irregular with undulated margin, mycelium raised at the centre, colony olivaceous black.
6.	Laevulose	49	+	Colony regular with entire margin, mycelium well developed, compact, massive and raised at the centre, colony dirty white.

Table 6 (Contd).

Nr. No.	Source of carbon.	Colony dia. in mm. after 8 days.	Sporulation.	Growth characters.
	II. Disaccharides.			
7.	Lactose.	45	++++	Colony irregular with undulated margin, mycelium thin, raised at the centre, colony olivaceous black.
8.	Sucrose	50	+++	Colony irregular with undulated margin, mycelium well developed, compact and raised at the centre, colony dark grayish olive.
	III. Polysaccharides			
9.	Dextrin.	58	++++	Colony circular with entire margin, mycelium well developed, compact and raised at the centre, colony dark, grayish olive.
10.	Inulin.	48	++	Colony circular with entire margin, mycelium well developed, compact, massive and raised at the centre, colony dirty white.

Table 6 (Contd).

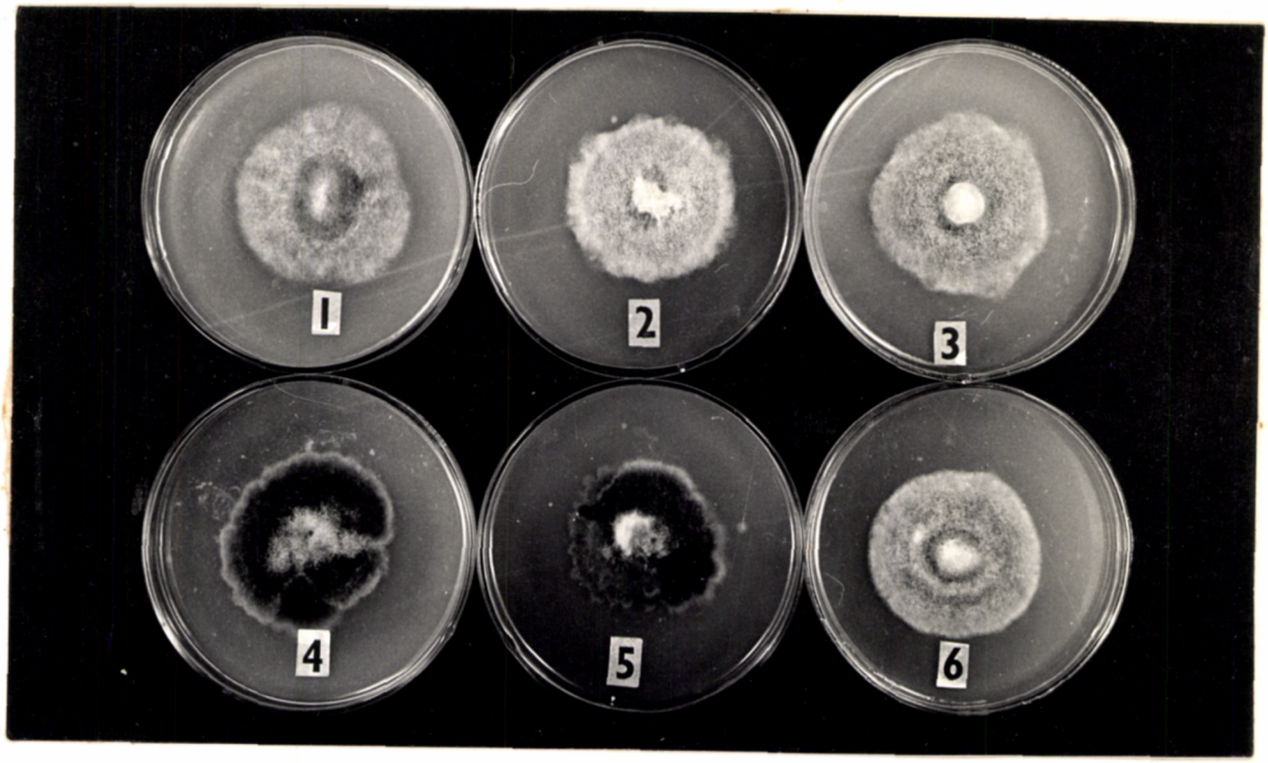
Sr. No.	Source of Carbon.	Colony dia. in mm. after 8 days.	Sporu-lation.	Growth characters.
11.	Control (without maltose)	15	-	Colony circular with entire margin, mycelium very thin with very poor growth, colony white.

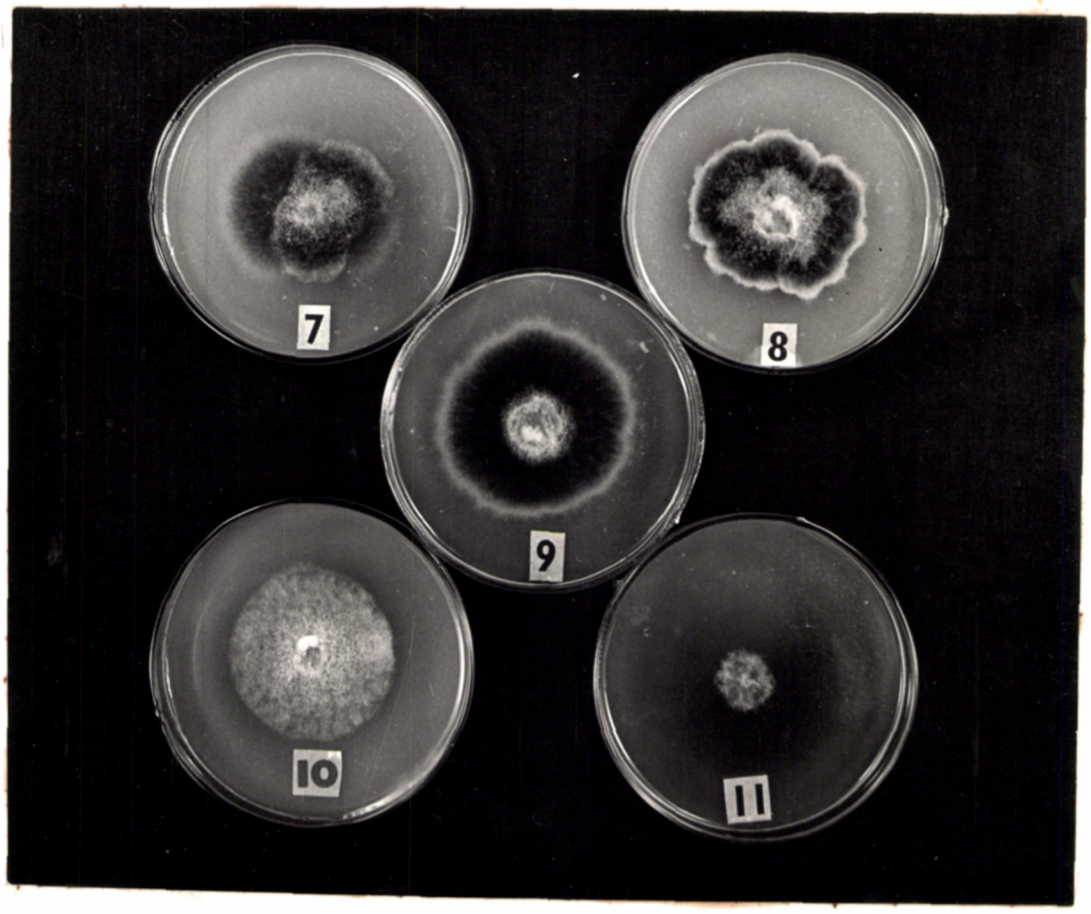
From the results given in the Table 6, it is observed that the fungus could utilize carbon, from almost all the carbon compounds. Abundant growth was observed on rhamnose, arabinose, galactose, laevulose, sucrose and dextrin, indicating that these carbon compounds are good carbon sources for the fungus growth, while D-xylose, glucose, lactose, inulin gave moderate growth. Fungus gave abundant sporulation on galactose, glucose, lactose and dextrin. Sucrose gave good sporulation whereas the fungus sporulated moderately on rhamnose and inulin and scanty on D-xylose, arabinose, laevulose.

Plate VI

1. Rhamnose.
2. D - xylose.
3. Arabinose.
4. Galactose.
5. Glucose.
6. Laevulose.
7. Lactose.
8. Sucrose.
9. Dextrin.
10. Inulin.
11. Control.
    (Coon's medium without maltose).

PLATE VI





C) Utilization of Nitrogenous compounds.

The following experiment was carried out to study the effect of inorganic and organic nitrogen compounds on growth of fungus and sporulation. Richards' agar without KNO_3 was used as a basal medium. The medium was distributed in 100 ml. lots in 250 ml. Erlenmeyer Flasks. Different inorganic and organic nitrogenous compounds calculated on basis of nitrogen contents equal to those of KNO_3 were added in Richards' agar, without KNO_3 served as control. This medium was sterilized, duplicate plates were poured, inoculated at the centre with equal fungus bits obtained by cutting with a cork-borer from a young growing culture and incubated at room temperatures (20^0-28^0 C). After 6 days of incubation colony diameter, growth characters as well as sporulation were recorded. The results are given in the Table 7 (Plate VII.)

The following nitrogenous compounds were used for the test.

(a) Inorganic nitrogenous compounds:

1. Ammonium nitrate.
2. Ammonium tartarate.
3. Magnesium nitrate.
4. Potassium nitrate.
5. Sodium nitrate.

(b) Organic nitrogenous compounds:

1. Egg - albumin.

2. Gelatin.

3. Urea.

4. Control (Richards' midium without KNO_3).

Table 2.

Utilisation of nitrogen compounds by Union alternaria.

Sr. No.	Source of Nitrogen.	Mean colony dia. in mm. after 8 days.	Sporulation.	Growth characters.
	(a) Inorganic compounds.			
1.	Ammonium nitrate.	32	-	Colony irregular with undulated margin, mycelium thick and compact, colony safrano pink.
2.	Ammonium tartarate.	45	-	Colony irregular with undulated margin, mycelium thick with aerial hyphae, colony avellaneous.
3.	Magnesium nitrate.	57	-	Colony irregular with undulated margin, mycelium thick, compact with aerial hyphae, colony safrano pink.

Table 7 (contd).

Sr. No.	Source of Nitrogen.	Mean colony dia. in mm. after 8 days.	Sporulation.	Growth characters.
4.	Potassium nitrate.	44	-	Colony circular with entire margin, mycelium thick and compact, colony dirty white.
5.	Sodium nitrate.	47	+	Colony circular with entire margin, mycelium thick, compact and raised at the centre, colony light olive gray.
	b) Organic compounds.			
6.	Egg-albumin.	75	++++	Colony circular with entire margin, mycelium very thin with aerial hyphae, colony dark olive gray.
7.	Gelatin.	48	-	Colony irregular with undulated margin, mycelium thick, compact with poor aerial hyphae and raised at the centre, colony puritan gray.

Table 7 (contd).

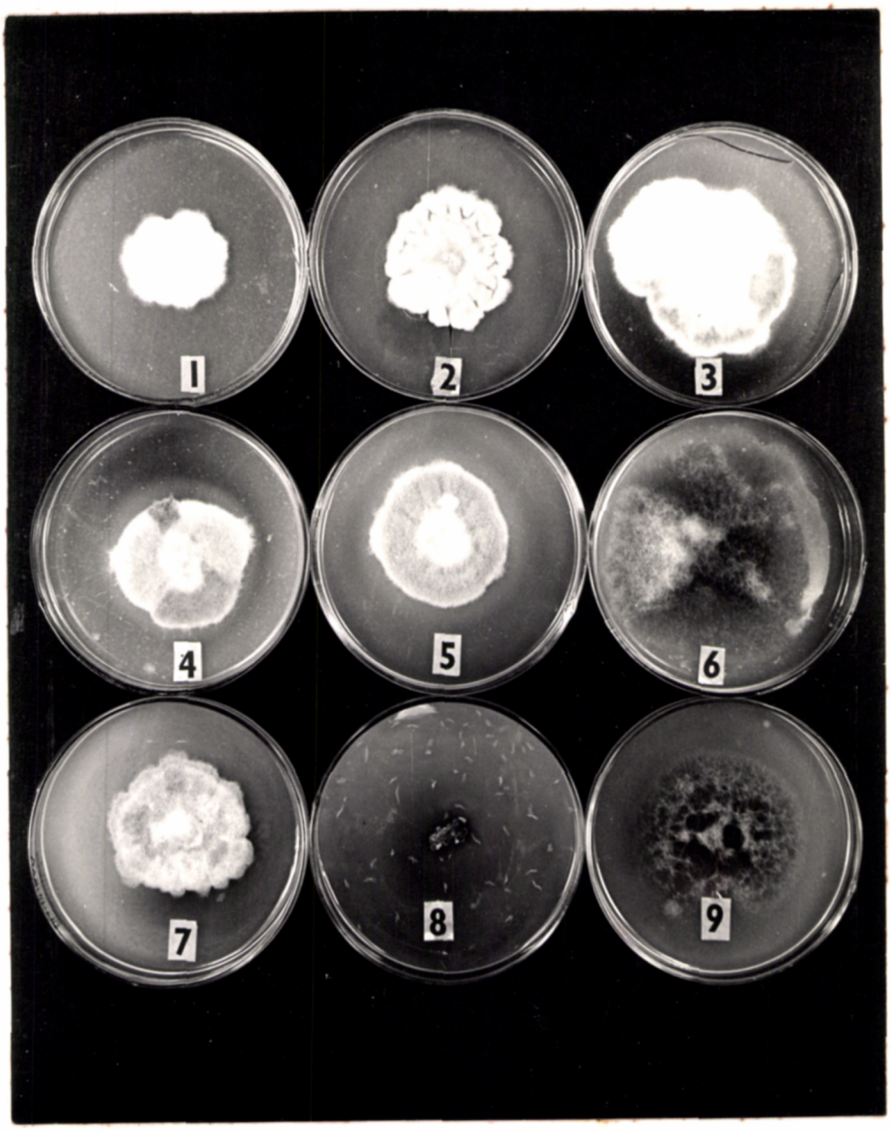
Sr. No.	Source of Nitrogen.	Mean colony dia.in mm.after 8 days.	Sporu-lation.	Growth Characters.
8.	Urea	-	-	No Growth.
9.	Control (Richards' medium without KNO_3)	86	+	Colony circular with entire margin, myce-lium very thin with aerial hyphae, colony olive gray.

Sporulation:- ++++ = Abundant;
+++ = Good;
++ = Moderate;
+ = Scanty;
- = Nil.

From the results given in the Table 7, it is seen that the fungus could grow profusely on ammonium tartarate, magnesium nitrate, potassium nitrate, sodium nitrate, egg-albumin, gelatin. Moderate growth was observed on ammonium nitrate. There was no growth at all on Urea.

Abundant sporulation was obtained on egg-albumin and scanty on sodium nitrate.

PLATE VII



1) Ammonium nitrate
2) Ammonium tartarate
3) Magnesium nitrate
4) Potassium nitrate
5) Sodium nitrate
6) Egg - albumin
7) Gelatin
8) Urea
9) Control (Richards' medium without KNO_3)

D) Enzyme Production

The enzymatic activity of the fungus as evidenced by the production of extracellular enzymes in culture media, was studied as per method described by Crabill and Reed (1915). A basal medium of the following compositions was prepared.

1) Magnesium sulphate	....	0.5 gm.
2) Potassium di-hydrogen phosphate	....	1.09 gm.
3) Potassium chloride	....	0.5 gm.
4) Ferrous sulphate	....	Trace.
5) Agar	....	30 gm.
6) Water	....	1000 ml.

The above medium was prepared and distributed in 100 ml.lots in 250 ml. Erlenmeyer flasks. The following zymolytes were added separately.

1. Starch
2. Egg- albumin.
3. Inulin
4. Gelatin.
5. Casein.

The medium was sterilized and duplicate plates were poured with each zymolyte. Plates were inoculated and incubated at room temperatures (25° to 29° C.) for 8 days. Uninoculated plates served as a control in each case. The production of enzymes was judged either by colour reaction or by ability of the fungus to grow on a particular compound, which was added as a sole

source of energy in the medium. These res summarised below and recorded in the Table

1) Diastase: Two grams of potato starch to 100 ml. of the basal medium. Fungus mad growth and produced distinct "halo" around fungus colony when it was treated with iodine solution (one per cent). This clearly showed the ability of the fungus to hydrolyse starch by production of diastase.

2) Trypsin: One gram of egg albumin was added to 100 ml.of basal medium, the medium was then sterilised at 15 lbs.pressure for 30 minutes for the coagulation of the protein. The media supported good growth of the fungus showing its ability to produce the enzyme trypsin.

3) Inulase: One gram of inulin was added to 100 ml. of the basal medium. The fungus grew well on this medium, indicating its ability to produce enzyme inulase.

4) Gelatinase: One gram of gelatin was added to 100 ml. of the basal medium. The fungus grew well on this medium and when colonies were treated with acidified mercuric chloride solution "halo" was produced around the colonies, indicating the ability of the fungus to produce the enzyme gelatinase.

5). Erepsin: One gram of casein was added in 100 ml. of the basal medium, in order to prepare casein agar. The fungus showed good growth on this medium and

produced clear zone around this colony, showing its ability to produce the enzyme erepsin.

Table 8.

Production of Enzymes by alternaria sp.

Sr. No.	Medium	Mean Colony dia. in mm. after 8 days.	Sporulation.	Enzyme produced	Intensity of enzyme production.
1.	Potato Starch agar.	75	Good	Diastase	+++
2.	Egg-albumin agar.	65	Abundant	Trypsin	+++
3.	Inulin agar.	60	Good	Inulase	++
4.	Gelatin agar.	55	Moderate	Gelatinase	++
5.	Casein agar.	55	Good	Erepsin	++

Intensity of enzyme production :

Abundant = ++++

Good = +++

Moderate = ++

Scanty = +

Nil = -

From the results given in the Table 8, it indicate that the fungus could produce a variety of enzymes as could be judged by the growth andthe tests. The fungus produced diastase, inulase, gelatinase, erepsin and trypsin in large quantities.

4) GROWTH OF THE FUNGUS IN RELATION TO HYDROGEN-ION CONCENTRATION.

Uppal et al (1938) studied cumin blight caused by Alternaria burnsii and stated that fungus could grow in a wide range of hydrogen-ion concentration.

Arya and Prasada (1962) studied Alternaria blight of linseed and stated that the fungus showed a wide range of pH ranging from 3.0 - 8.5 ; the optimum being 5.0 to 6.8.

Richards' medium was prepared and distributed in 100 ml. lots in 250 ml. of Erlenmeyer flasks. pH was adjusted colorimetrically with the help of N/10 sulphuric acid and or N/10 sodium hydroxide solution. The test tubes filled with the medium for each pH value were sterilised along with the flask at 15 lbs. pressure for 15 minutes. After sterilisation the exact pH was then determined with the help of Beckman's pH meter with glass electrode by testing the sample solution from the tubes. The flasks were then inoculated with the fungus bit and incubated at room temperatures (26^{0} to 28^{0} C.) for 21 days.

The mycelial mat was separated from the solution with the help of filter paper and dried at 40^{0}C. until constant weight was obtained.

The weights of the dried mycelial mat are given in Table 9.

Table 9.

Effect of H-ion concentration on Alternaria sp.

Sr. No.	pH of the medium		Dry weight of the mycelial mat after 21 days. (in mg.)
	Before Sterili-zation.	After Sterili-zation.	
1.	2.5	3.3	No growth.
2.	3.5	3.4	No growth.
3.	4	4.3	1570
4.	5	5.1	1740
5.	6	6.2	1730
6.	6.5	6.3	1690
7.	7	7.3	1680
8.	7.5	7.5	1605
9.	8	8.0	1580
10.	9	8.8	1550
11.	10	9.6	1470
12.	10.5	10.2	1300

It is seen from the Table 9, that the fungus could grow well within a wide range of pH. The maximum growth was obtained at pH 5.1. It could also grow well between 4.3 and 8.8 . It could also be noticed that the fungus had tendancy to grow better in acidic media.

F) Thermal Death Point.

10 ml. of the Coon's liquid medium was filled in the tubes having uniform shape and size. These tubes were then sterilized and then inoculated with a small fungus bit from a young growing culture. The inoculated tubes were immediately kept in the water bath, at the constant temperature of 35°, 40°, 45°, 50°, 55°, 60°, 65°, and 70° C. for 10 minutes. The water in the water bath was constantly kept stirred in order to keep the uniform temperature of water. A test tube with Coon's liquid medium was used for keeping the thermometer for recording temperature. After a exposure for 10 minutes, at a particular temperature, the tubes were immediately placed in cold water and then incubated at room temperatures (23° to 28°C.). There was no growth in the tubes subjected to the temperatures 60°, 65° and 70° C; while in the remaining test tubes growth was observed. In order to find out exact Thermal-death-point of the fungus, the above procedure was repeated for the following temperatures viz; 56°, 57°, 58°, and 59°C. The results are given in the Table 10.

Table 10.

Thermal-death-point of the fungus.

Sr. No.	Temperature in °C.	Fungus growth.
1.	35	Abundant growth was observed after 2 days.
2.	40	Abundant growth was observed after 2 days.
3.	45	Abundant growth was observed after 3 days.
4.	50	Moderate growth was observed after 5 days.
5.	55	Poor growth was observed after 7 days.
6.	56	No growth was observed after 8 days.
7.	57	- do - -do- -do-
8.	58	- do - -do- -do-
9.	59	- do - -do- -do-

The above results indicate that the Thermal death point of the fungus is 56 °C.

CHEMICAL CONTROL.

A) EFFECT OF THE DIFFERENT FUNGICIDES ON THE ONION ALTERNARIA IN VITRO.

The important object of any plant pathological investigation aims at finding out suitable control measures amongst which evolving a resistant variety is the best remedy. But in the absence of resistant varieties, the next best measure is to resort to chemical method of control either used as prophylactic or as curative. Before recommending any chemical it is very essential to test it *in vitro* and also *in vivo* to find out its efficacy, dosage, time of treatment and its economics.

The following fungicides were tried for the study.

1. Aureofungin.
2. Blitox-50.
3. Captan.
4. Cuman.
5. Dithane Z-78.
6. Duter.
7. Kasumin.
8. Benlate.
9. Kitasin.
10. Vitavax.
11. Difolitan.
12. Hinosan.

Coon's medium was prepared, and distributed in 100 ml. lots in 250 ml.of flasks and sterilized at 15 lbs. pressure for 15 minutes. Different quantities of the fungicides to be tested were added to each of these flasks. To ensure even distribution of the fungicides the flasks were shaken vigorously and media were poured in petridishes, for each fungicide. After solidification, the plates were inoculated with the fungus culture and incubated for 8 days at room temperatures of 25° -28° C. Coon's medium without any fungicide in petri plate served as control. Observations on the colony diameter and sporulation recorded after 8 days of incubation are given in Table 11. (Plate VIII).

Table 11.

Effect of the different fungicides on the growth and sporulation of the fungus.

Sr. No.	Name of the fungicide.	Chemical nature.	Concen-tration used.	Colony dia. in mm. after 8 days.	Sporu-lation.
1.	Aureofungin	N-methyl p-amino-acetophenone	2.2 mg/ 100 cc.	12	++++
2.	Blitox-50	50% meta-llic copper.	200 mg/ 50 cc.	26	++

Table 11 (contd).

Sr.No.	Name of the fungi-cide.	Chemical nature.	Concen-tration used.	Colony dia.in mm. after 8 days.	Sporu-lation.
3.	Captan	83% N-Trichoro-methyl-thiotetra hydrophthalimide	200 mg/ 50 cc	11	-
4.	Cuman	80% zinc dimethyl dithio-carbamate.	100 mg/ 50 cc.	6	-
5.	Dithane Z-78	78% zinc ethylene bisdithio carba-mate	100 mg/ 50 cc.	35	-
6.	Duter	Triphenyltin hydroxide.	100 mg/ 50 cc.	19	+
7.	Hasumin	Developed from actinomyces *Streptomyces hasygaensis	10 mg/ 100 cc.	48	++
8.	Benlate	Methyl 1 - (butylcarbamoyl) -2-benimidazole-carbamate.	5 mg/ 100 cc.	55	+
9.	Kitazin	O,O-Diisopropyl-S-benzyl thiophosphate.	0.12 cc/No 100 cc. growth		-

Table 11 (contd)

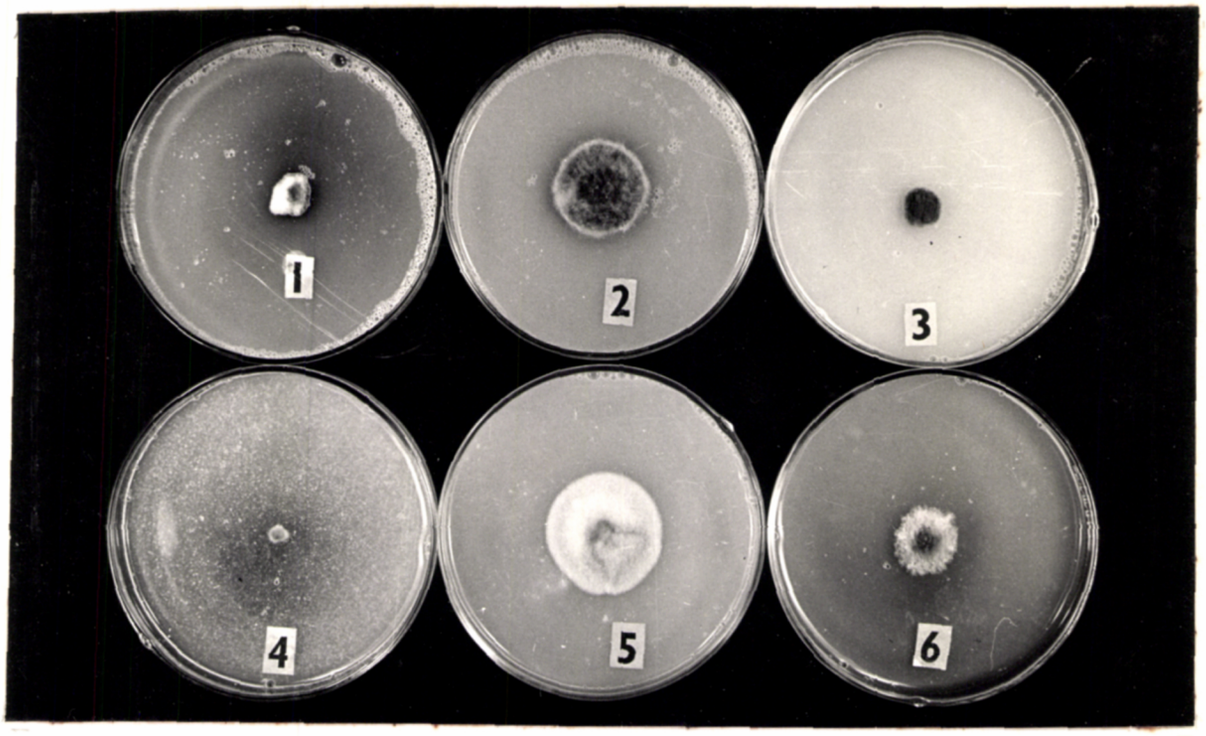
Sr. No.	Name of the fungicide.	Chemical nature.	Concen-tration used.	Colony dia.in mm. after 8 days.	Sporu-lation.
10.	Vitavax	2,3-Dihydro-5-Caroboxanilide-6-methyl-1,4-oxathiin.	5 mg/ 100 cc.	10	-
11.	Difoliton	N(1,1,2,2-tetrachloro-ethyl) thio-4-cyclohexene-1,2-dicarboxi-mide.	10 mg/ 100 cc.	8	-
12.	Hinosan	O-ethyl-S,S diphenyl dithiophosphate	0.5 mg/ 100 cc.	13	-
13.	Control (Coon's medium only).	-	-	60	++++

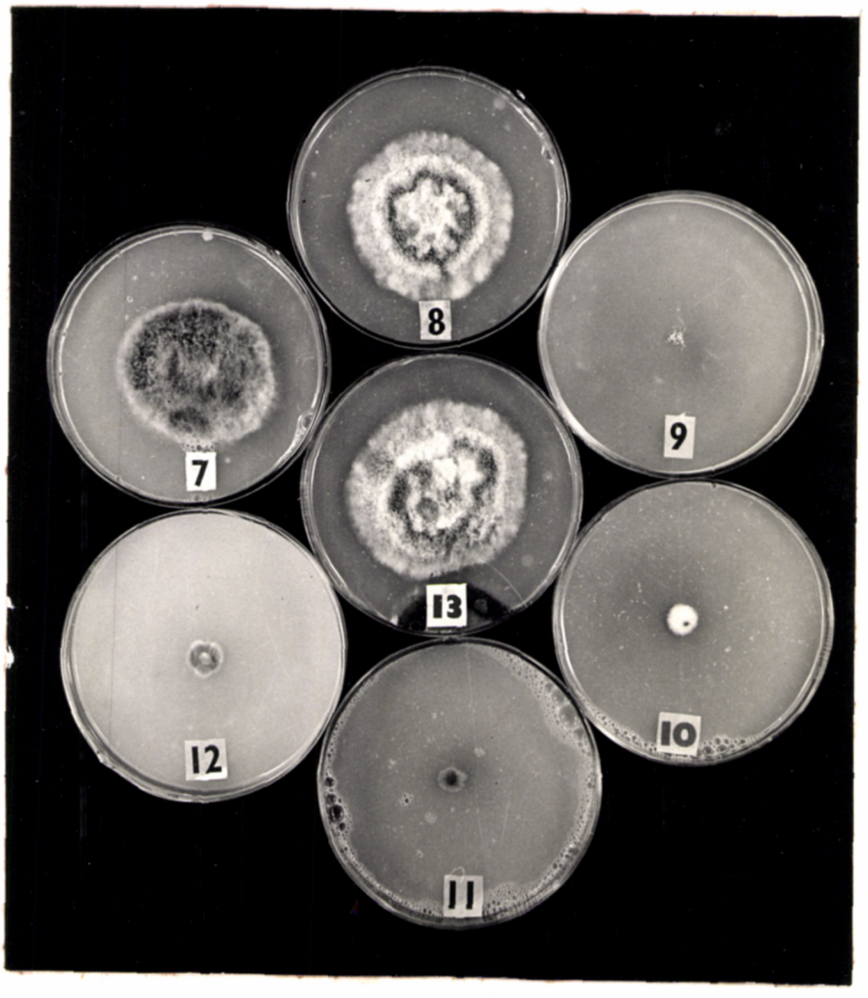
It will be seen from the results given in the Table II, that the fungus made good growth on Kasumin and benlate, moderate on blitox-50 and dithane -78 Fungus did not grow well on aureofungin, captan, cuman, duter, vitavax, difoliton and hinosan. In kitazin, the fungus made no growth at all. The sporu-lation was abundant in aureofungin, moderate in bli-tox-50 and Kasumin and scanty on duter and benlate. There was no sporulation in captan, cuman, dithane -78, duter, kitazin, vitavax, dofoliton and hinosan. It can, therefore, be concluded that kitazin was the best chemical which prevented growth and sporulation of the fungus followed by cuman, difoliton, vitavax, captan, hinosan, duter, aureofungin , blitox- 50.

Plate VIII

1. Aureofungin.
2. Blitox - 50.
3. Captan.
4. Cuman.
5. Dithane Z-78.
6. Duter.
7. Kasumin.
8. Benlate.
9. Kitazin.
10. Vitavax.
11. Difoliton.
12. Hinosan.
13. Control.
    (Coon's medium only)

PLATE VIII





3). [illegible] Used as a Prophylactic Measure.

This experiment was undertaken to see the efficacy of some fungicides tested earlier in vitro test, on the control of disease on onion plants when used as a prophylactic measure. This would help to recommend the effective fungicide to control a disease in the field.

For this purpose, three seedlings of onion were raised in 9" pots. When the plants were 2½ months old, they were sprayed with the chemicals and on the next day the pots were kept in moist chambers for 24 hours. The plants were then inoculated by spraying of the fungus and again were maintained under the moist chambers for 24 hours. Adequate control was provided without fungicidal spray. Observations on the incidence of disease were noted after 15 days of inoculation, recording number of spots and per cent leaf area infected and given in the Table 12.

Table 12.

Sr. No.	Name of Chemical.	Dose per acre recommended.	Percentage of leaves infected.	Average percentage of leaf area affec-ted.
1.	Aituzin	375 cc/ 320 litres.	8	3.6
2.	Duman	908 gms/ 450 litres.	8.5	4.5

Table 12 (Contd).

Sr. No.	Name of chemical.	Dose per acre recommended.	Percentage of leaves infected.	Average percentage of leaf area affected.
3.	Difoliton	200 gms/ 200 litres.	9.2	5.0
4.	Vitavax	175 gms/ 360 litres.	9.8	6.0
5.	Captan	1.816 gms/ 450 litres.	10.0	7.5
6.	Minosan	160 cc/ 320 litres.	10.6	10.0
7.	Dithane-Z-78	908 gms/ 450 litres	11.5	13.0
8.	Aureofungin.	4 gms/ 160 litres.	13.0	16.0
9.	Control		25.0	19.0

The results given in the Table 12, indicate that almost all chemicals showed good control of the disease. There was 25 per cent of leaf infection in control plants (without chemical spray). As against this, per cent leaf infection was brought down to 8 to 13, when chemicals

were used. In control, 19.0 per cent of the leaf area was found affected but by spraying chemicals, it was brought down to 3.5 to 16 per cent. Among the chemicals tested, Aitazin proved to be the best chemical in checking the incidence of the disease. Cumun, difolaton, vitavax, captan, hinosan, dithane---78, aureofungin were next in order of their mention.

Taxonomy and Nomenclature.

Nees (1816) described the genus Alternaria to include fungi with obclavate, coloured and muriform spores borne in chains. The chief distinguishing feature between Macrosporium and Alternaria, was the catenulation or chains of spores which the latter produces under favourable conditions. Fries (1825) did not recognise the genus Alternaria but included it under Torula. Later, Chevallier (1826) separated the genus Alternaria and stated its differentiating characters from Torula as 'links' of spores of Alternaria separated by filiform connections.

Wallroth (1833) established the genus Stemphylium which was closely allied to Alternaria but in Alternaria the spores were borne in chains and in Stemphylium they were not so. Stemphylium spores were more oblong, constricted at the middle and more or less packet shaped or sarcinae form, as against Alternaria spores which were obclavate, tapering towards the upper end and forming a more or less elongated beak.

In delimitation of the species in the genus Alternaria the different characters like shape, size, septation, colour of the spores, length of the beak and conidiophore measurements and its specifications are of major importance. Other characters like habitat, sympotoms, mycelium and host range are also considered

in delimiting its specific rank. Due to environmental conditions, however, these characters are very variable.

In the light of this, it becomes necessary to compare morphological characters of the fungus on hand with the fungi reported on onion before the specific name is assigned. This comparison is given in the Table 13.

Table 13.

Comparison between species of Alternaria affecting Allium Sepa L.

Alternaria species.	Conidiophores in µ	Conidia in µ	Authority.
A. palandhui Ayyangar.	-	10.5-77 X 3.5-14.	Ayyangar-1938.
A. allii Nolla	20-180 X 4-18	106-320X 12-24	Nolla -1927
A. porri(Ell.) Neerg.	27-45 in length.	106-220X 17.5-26	Angell - 1929.
A. cepulicola Rao, V.G.	29.4-90.3 X 8.4-12.6	58.8-184.8 X 21-46.2	Rao, V.G. - 1963.
A. sp.under study (1970-71)	21.84-96.80 X 5.13-9.76	46.60-110.08 X 10.44-29.84	

From the Table 13, it can be seen that the fungus on hand mostly agrees with *A. sapulicola* on basis of morphological characters. Although slight variations in these characters, do not warrant to create a new variety for the fungus on hand. Moreover, *Alternaria* is considered to be a weak parasite becoming prominent whenever environmental conditions become favourable besides the availability of the desired or allied suscepts. In view of this the fungus inciting leaf spot on onion in Maharashtra has been identified as *A. sapulicola* Rao, V.G.

[illegible] and conclusion.

The present work deals with the investigations on blight disease of onion incited by a fungus parasite of the genus Alternaria.

Leaf spot on onion was common in rabi around Poona. Cloudy weather coupled with alternate rainfall and sunshine in the field resulted in increasing the incidence of the disease.

The disease manifested itself on leaves only in the form of dark-purplish brown, oval to irregular, scattered spots with a paler outer zone, resulting in blight and withertip, under favourable environmental conditions. No other part of the onion plant was affected. The pathogen was readily obtained in pure culture and its pathogenicity established. Besides onion, it could infect bhuimi also. None of the varieties tested proved tolerant or immune. The morphological characters of conidia, conidiophores were studied and described. The conidia were obclavate to muriform with short beaks and has variations in the measurements from the host and the culture medium. The measurements of conidia, conidiophores and other characters closely agreed with Alternaria nagulinoia and pathogenic on onion. It could show differences in some of the characters. These differences were

not so critical, to warrant creation of a new variety. As such, the fungus has been assigned the binomial A.capulincola Rao, V.G.

The cultural, physiological studies of the pathogen revealed that it could grow and sporulate on a variety of media, could utilise some of the carbon and nitrogen compounds, had optimum tempera-ture range from 25° to 28° C., tolerate pH 4.3 to 10.2 and produce enzymes diastase, inulase, gelatinase, erepsin and trypsin in varying quantities. Thermal death point of the fungus was 56° C.

In vitro tests, the fungus was inhibited by kitazin, cuman, difoliton, vitavax, capta n, hinosan, cuter, blitox-50 and an antibiotic aureofungin by the recommended concentrations. Most of the chemicals had reduced 50 per cent infection on leaves when used on plants as prophylactic measure. Among the fungicides, kitazin proved superior to all other fungicides in vivo and in vitro tests.

SUMMARY.

Leaf blight disease of onion (Allium cepa L.) incited by Alternaria was studied and described. The fungus could incite symptoms only on the leaves. Morphlogical, cultural and physiological characters were studied and described. The pathogen could infect bhandi only. None of the onion varieties tested was tolerant or immune.

The pathogen was identified on basis of the morphological characters as A.nepulicola.

In vitro, kitazin, cuman, difoliton, vitavax, captan, hinosan, duter, blitox-50 and aureofungin effectively inhibited the growth of fungus. In vivo, most of the chemicals prevented 50 per cent of the infection on leaves, but kitazin proved superior among them.

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