VARIABILITY OF *Alternaria* ISOLATES CAUSING LEAF BLIGHT DISEASES IN CUCURBITS

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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Faculty of Agriculture
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DECLARATION

I, hereby declare that the thesis entitled "**Variability of *Alternaria* isolates causing leaf blight diseases in cucurbits**" is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that the thesis entitled **"Variability of *Alternaria* isolates causing leaf blight diseases in cucurbits"** is a record of research work done independently by **Ms. Resmi. A. R.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CONTENTS

CHAPTER	TITLE	PAGE NO.
1	INTRODUCTION	1-2
2	REVIEW OF LITERATURE	3-20
3	MATERIALS AND METHODS	21-29
4	RESULTS	30-78
5	DISCUSSION	79-90
6	SUMMARY	91-94
	REFERENCES	i - x
	APPENDIX	
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Colony characters of different isolates of *Alternaria*	34
2	Sporulation of different isolates of *Alternaria*	36
3	Effect of selected media on the growth rate of *Alternaria* isolate from ash gourd	37
4	Effect of selected media on the growth rate of *Alternaria* isolate from snake gourd	38
5	Effect of selected media on the growth rate of *Alternaria* isolate from ridge gourd	40
6	Effect of selected media on the growth rate of *Alternaria* isolate from bottle gourd	41
7	Effect of selected media on the growth rate of *Alternaria* isolate from bitter gourd	42
8	Effect of selected media on the growth rate of *Alternaria* isolate from ivy gourd	44
9	Effect of selected media on the growth rate of *Alternaria* isolate from pumpkin	45
10	Growth in days taken by *Alternaria* isolates on different media	46
11	Size of conidial chain of *Alternaria* isolates	49
12	Morphological descriptions of different isolates of *Alternaria* from different cucurbitaceous vegetables	50-53
13	Morphological characters of different isolates of *Alternaria* from Vellanikkara	54
14	Morphological characters of different isolates of *Alternaria* from Elanad	55
15	Dissimilarity matrix of *Alternaria* isolates from the two locations based on morphological characters	56
16	Grouping of *Alternaria* isolates from the two locations based on dissimilarity index for morphological characters	56
17	Identified *Alternaria* spp. from different locations	58
18	Effect of temperature on fresh and dry weight of isolates of *Alternaria* from Vellanikkara	59
19	Effect of temperature on fresh and dry weight of isolates of *Alternaria* from Elanad	61
20	Effect of p^H on fresh and dry weight of isolates of *Alternaria* from Vellanikkara	63

Table No.	Title	Page No.
21	Effect of p^H on fresh and dry weight of isolates of *Alternaria* from Elanad	65
22	Effect of light on fresh and dry weight of isolates of *Alternaria* from Vellanikkara	66
23	Effect of light on fresh and dry weight of isolates of *Alternaria* from Elanad	68
24	Effect of carbon sources on fresh and dry weight of isolates of *Alternaria* from Vellanikkara	70
25	Effect of carbon sources on fresh and dry weight of isolates of *Alternaria* from Elanad	71
26	Effect of nitrogen sources on fresh and dry weight of isolates of *Alternaria* from Vellanikkara	73
27	Effect of nitrogen sources on fresh and dry weight of isolates of *Alternaria* from Elanad	75
28	Per cent germination of conidia of different isolates of *Alternaria*	77

LIST OF FIGURES

Figure No.	Title	Between pages
1	Conidia and conidiophores of *A. alternata* of ash gourd isolate from Vellanikkara	55-56
2	Conidia and conidiophores of *A. alternata* of snake gourd isolate from Vellanikkara	55-56
3	Conidia and conidiophores of *A. alternata* of ridge gourd isolate from Vellanikkara	55-56
4	Conidia and conidiophores of *A. alternata* of bottle gourd isolate from Vellanikkara	55-56
5	Conidia and conidiophores of *A. tenuissima* of bitter gourd isolate from Vellanikkara	55-56
6	Conidia and conidiophores of *A. tenuissima* of ivy gourd isolate from Vellanikkara	55-56
7	Conidia and conidiophores of *A. alternata* of pumpkin isolate from Vellanikkara	55-56
8	Conidia and conidiophores of *A. alternata* of ash gourd isolate from Elanad	55-56
9	Conidia and conidiophores of *A. tenuissima* of snake gourd isolate from Elanad	55-56
10	Conidia and conidiophores of *A. alternata* of ridge gourd isolate from Elanad	55-56
11	Conidia and conidiophores of *A. alternata* of bottle gourd isolate from Elanad	55-56
12	Conidia and conidiophores of *A. tenuissima* of bitter gourd isolate from Elanad	55-56
13	Conidia and conidiophores of *A. alternata* of ivy gourd isolate from Elanad	55-56
14	Conidia and conidiophores of *A. alternata* of pumpkin isolate from Elanad	55-56
15	Unweighed pair group average method (UPGMA) dendrogram based on morphological characters of *Alternaria* isolates from the two locations	56-57

LIST OF PLATES

Plate No.	Title	After Page No.
1	General symptom of *Alternaria* leaf blight in cucurbits	1
2	View of artificial inoculation of the pathogen	22
3	Symptomatology of *Alternaria* leaf blight diseases of cucurbits-Under natural condition	32
4	Symptomatology of *Alternaria* leaf blight diseases of cucurbits-Under artificial condition	33
5	Cultural characters	39
6	Morphological characters	48
7	Conidia of various *Alternaria* isolates from Vellanikkara	55
8	Conidia of various *Alternaria* isolates from Elanad	55
9	Conidial germination and histopathology	77
10	Host range	78
11	Effect of toxin on pathogenesis of leaf blight diseases of cucurbitaceous vegetables	78

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Introduction

1. INTRODUCTION

Cucurbits are important vegetable crops cultivated extensively in the tropics for their edible fruits, which are consumed either raw or as cooked vegetable. Cucurbitaceous vegetables include cucumbers, melons, pumpkins and gourds. They possess an important role in human diet. They are valuable because of their nutritive and medicinal properties. Fruits are rich source of Vitamin A, B and C and minerals like iron and calcium. Some of the members like bitter gourd and ivy gourd have got therapeutic value, particularly in the treatment of diabetics, bronchitis and skin diseases. They all belong to the family cucurbitaceae and are grown during summer. They are climbing or prostrate herb, growing throughout India, possesses glabrous stem, long tap root system and ovoid, round or elliptical fruits.

Cucurbits are prone to the attack of many diseases. Among the various diseases *Alternaria* blight is one of the serious diseases (Plate 1). A large number of species of *Alternaria* are known to cause leaf blight diseases, some of which are economically very important. While many species of *Alternaria* have got wide host range, some show a certain degree of host specificity. It causes diseases on various cucurbits and cause severe yield losses. The disease cause severe damage to leaves and reduce vigour and yield of the plant. In chillies upto 85% losses were reported due to this disease. The yield losses were reported in various cucurbits such as 77.7 % (bitter gourd), 65.3% (bottle gourd), 80% (pumpkin), 69.1% (ridge gourd) and 88.3% (watermelon) by Khandelwal and Prasada (1979).

Several *Alternaria* species have been reported to cause the disease from different parts of the world. Among these *A. cucumerina*, *A. tenuissima* and *A. alternata* are the most prominent ones reported in cucurbits. Chahal *et al.* (1970) reported *Alternaria* leaf spot of cucurbits caused by *Alternaria cucumerina* from Punjab. A leaf spot disease of melon caused by *Alternaria alternata* f.sp. *cucurbitae* was reported by Vakalounakis (1990a). *Alternaria tenuissima* (Kumar *et al.*, 2001) and *Alternaria* *alternata* (Davis, 2003) was reported as pathogens causing leaf blight disease on ivy gourd.

The perusal of literature revealed not much information about the diseases of cucurbits by *Alternaria* from Kerala although many reports were available on the occurrence of leaf blight diseases on these crops from different parts of India. From the early reports cucurbits are known to be infected by many species of *Alternaria*, but documentation on the variability existed among them are not available. In this context the present study is taken up, which will give more information about leaf blight diseases of cucurbits and the variability if any, existing among the different isolates of *Alternaria* from different cucurbitaceous hosts. The research programme entitled "Variability of *Alternaria* isolates causing leaf blight diseases in cucurbits" envisaged the following aspects.

1. Isolation of *Alternaria* sp. associated with leaf blight diseases of cucurbitaceous vegetables.

2. Symptomatology of the disease.

3. Cultural and morphological characters of *Alternaria* sp.

4. Physiological and nutritional characters of *Alternaria* sp.

5. Identification of different *Alternaria* sp. isolated.

6. Host range of the different *Alternaria* sp. isolated.

7. Effect of toxin of *Alternaria* on disease development.

Review of Literature

2. REVIEW OF LITERATURE

Cucurbits form an important and a big group of vegetable crops cultivated extensively in the tropics. This group consists of a wide range of vegetables, either used as salad (cucumber) or for cooking (all the gourds) or for pickling (cucumber) or as desert fruits (muskmelon and watermelon) or candied or preserved (ash gourd). They have tremendous economic use as food plants and are also valuable because of their nutritive and medicinal properties. Several diseases especially leaf and fruit spots are noticed in many vegetable fields where it is cultivated in a large scale. *Alternaria* is an important pathogen causing leaf blight disease in cucurbits and solanaceous crops. Several reports were available on *Alternaria* leaf blight of cucurbitaceous crops from different parts of India (Chahal *et al.* (1970); Narain *et al.* (1985); Bhargava and Singh (1992) and Ahamad and Narain (2000). Whereas perusal of literature revealed very few reports on *Alternaria* leaf blight of cucurbits from Kerala (Davis, 2003).

2.1 SYMPTOMATOLOGY

An *Alternaria* leaf spot of cucumber, watermelon and muskmelon caused by *Alternaria cucumerina* was reported by Chahal *et al.* (1970) from Ludhiana. They recognized the disease in the field by the presence of olivaceous coloured circular to elliptical spots on matured leaves. They observed that the young and actively growing leaves were almost free from the disease or infected with very small necrotic spots. The severely infected leaves showed burning effect, which ultimately dried and fell prematurely. They also reported the presence of olive green mass of conidia on the well advanced stage of spots.

In a study of *Alternaria* blight of cucurbits from Rajasthan, Bhargava and Singh (1985) described the symptoms produced on watermelon. They reported that the symptoms started as yellow or brown spots with bright yellow halo and later became larger with frequent zonations and marginal necrosis.

A severe leaf spot disease of cucumber caused by *Alternaria alternata* was first observed by Vakalounakis and Malathrakis (1988) in Greece. They observed that the lesions ranged from the size of pinprick to five centimeter in diameter with necrotic tissues surrounded by yellow zone. Later in 1989, Vakalounakis noticed an infectious leaf spot disease caused by *Alternaria alternata* f.sp. *cucurbitae* in green house crops of *Cucumis sativus* in Greece. He observed the symptoms appeared in late autumn, on leaves of mid and upper parts of the plant, as necrotic flecks surrounded by chlorotic halos and later enlarged to form circular lesions bearing brown black fruiting bodies of the pathogen. Again Vakalounakis (1990a) reported the same pathogen, *Alternaria alternata* f.sp. *cucurbitae* on watermelon as leaf spot pathogen and described the symptoms. He reported that the symptom initiated as necrotic flecks with chlorotic halo on the cotyledons and the leaves of the middle and the upper parts of the plants. The flecks enlarged and coalesced to form lesions of two centimeter or more in diameter with brown fructifications of the pathogen.

Davis (2003) reported *Alternaria* leaf spot on ivy gourd. She described the symptoms developed on the leaves as dark brown lesions of size 2-5 cm with concentric zonations and a clear yellow halo on both surfaces of leaves. She also noticed severe infection on older leaves compared to younger ones. Usually the lesions coalesced affecting larger leaf area.

There were few reports on *Alternaria* infection on solanaceous crops and other vegetables. Small, irregularly shaped leaf lesions with dark brown centers surrounded by yellow margins caused by *Alternaria* species were observed by Chellemi and Mueller (1995) on tomato grown in the tomato producing regions of North Florida. Lesions began on the margins of older leaves, lacked any visible signs of concentric rings and eventually coalesced, resulting in defoliation. They reported that on artificial inoculation the symptoms developed on inoculated foliage seven days after inoculation.

The development of symptoms of *Alternaria capsici annui* on chilli was investigated by Basak and Chowdhary (1997) under artificial field conditions and noticed the development of similar type of symptoms that were produced under field conditions on nine days after inoculation. Under field conditions symptom developed from the margin of chilli leaves as water soaked lesions and later became brown with characteristic yellow halo

Pandey and Vishwakarma (1999) reported symptomatological variations in *Alternaria alternata* causing leaf blight in brinjal. They observed small, light brown, circular spots, all over the plant foliage in the initial stage of the disease. These spot were first initiated from the tips and margin of the leaf lamina progressing towards the midrib. Later, these spots coalesced within few days became irregular, larger in size and turned olivaceous black due to abundant sporulation. In 2002, Blodgett and Swart reported *Alternaria* leaf spot on *Amaranthus hybridus* and they observed brown to black, circular to oval, necrotic lesion only at the wound site of injured leaves.

2.2 ETIOLOGY OF LEAF BLIGHT DISEASES

Alternaria leaf blights of cucurbits are of common occurrence in India but literature revealed few reports from Kerala. A report on *Alternaria cucumerina* blight of watermelon causing serious losses throughout Rajasthan was given by Khandelwal and Prasada (1970). They also got the infection on muskmelon and cucumber by the same fungus in host range study. In the same year Chahal *et al.* (1970) recorded *Alternaria* leaf spot disease on various cucurbits *viz.*, muskmelon, cucumber, bottle gourd and watermelon for the first time from Ludhiana and identified the pathogen as *Alternaria cucumerina*. Ibrahim *et al.* (1975) also isolated *Alternaria cucumerina* from diseased leaves of watermelon for the first time from Egypt and they proved the pathogenicity of the isolate but observed variation in virulence when inoculated on cotyledons and true leaves of cotyledons.

A new leaf blight and fruit rot of watermelon caused by *Alternaria alternata* was reported by Naraian *et al.* (1985). A severe leaf spot disease of cucumber caused by *Alternaria alternata* was reported by Vakalounakis and Malathrakis (1988) from Greece.

Based on cultural and morphological characteristics, the fungus isolated from squash and muskmelon was identified as *Alternaria cucumerina* by Galiotti (1988) from Argentina. Later Bhargava and Singh (1992) reported from Jaipur, *Alternaria cucumerina* as the leaf blight pathogen on bottle gourd.

Bedi *et al.* (1993) isolated and reported *Alternaria alternata* from the infected leaves, tendrils and stems of watermelon from Punjab and pathogenicity was confirmed. *Alternaria alternata* f.sp. *cucurbitae* was isolated from seedling blight of bitter gourd and reported for the first time in Taiwan by Hwang *et al.* (1998). But *Alternaria cucumerina* was recorded on bitter gourd (*Momordica charantia*) by Ahamad and Narain (2000) for the first time from Uttar Pradesh.

A new leaf spot disease on *Coccinia grandis* was observed by Kumar *et al.* (2001) from Uttar Pradesh and the organism of the disease was identified as *Alternaria tenuissima.* But Davis (2003) reported *Alternaria alternata* as leaf spot pathogen on ivy gourd from Kerala. Narain *et al.* (2003) reported *Alternaria cucumerina* on cucurbitacous hosts, *Citrullus melo* var. *utilissimus* and *Citrullus melo* var. *momordica* from India. A leaf spot on watermelon and cucumber caused by *Alternaria alternata* f.sp. *cucurbitae* was reported from Hungary by Urbanzki *et al.* (2003).

Butler (1918) reported *Alternaria solani* on brinjal. The occurrence of an unidentified species of *Alternaria* on chilli leaves in India has been reported by Dutt (1937) and also by Edwards and Shrivastava (1951). Fruit rots of brinjal caused by *Alternaria tenuis, Alternaria solani* and *Alternaria tenuis* f.sp. *genuine* have also been reported from other countries by Neergard (1945) and Pucci (1947).

From India Vasudeva (1954) reported a leaf spot of brinjal caused by a fungus similar to *Alternaria tenuis* morphologically but not pathologically. Subramanian (1954) reported a strain of *Alternaria solani* causing fruit rot of chilli. The detailed description of the new species, *Alternaria melongenae*, and the influence of substrate on the spore size of the species, occurring on chilli and brinjal were reported by Rangaswami and Sambandam (1960a, 1960b and 1961).

Siddiqui (1962) isolated and studied *Alternaria tenuis* from *Solanum tuberosum*. A serious rot of bell peppers caused by *Alternaria* sp. was reported by Quebral and Shurtleff (1966) and found that eggplant and tomato were also susceptible to this fungus.

Sahi and Shyam (1993) isolated *Alternaria solani* and *Alternaria alternata* f.sp. *lycopersici* from leaf spots on tomato plants in Himachal Pradesh. A new *Alternaria* leaf blight disease on tomato was reported by Chellemi and Mueller (1995) from North Florida. But Behadli *et al.* (1997) from Iraq reported *Alternaria alteranata* f. sp. *lycopersici* as causal agent of canker disease of tomato.

First report of *Alternaria cassiae* on cowpea was given by Grange and Aveling (1998) from South Africa. Blodgett *et al.* (1999) reported *Alternaria tenuissima* as leaf pathogen of *Amaranthus hybridus*. *Alternaria alternata* on tomato, capsicum and spinash in Kumaon hills was reported by Bhatt *et al.* (2000).

Mondal *et al.* (2002) reported *Alternaria* leaf spot of grain amaranth. *Alternaria cassiae* as the causal agent of a new, destructive foliar disease of cowpea (*Vigna unguiculata*) was reported by van-den-Berg *et al.* (2003).

2.3 CULTURAL AND MORPHOLOGICAL CHARACTERS OF *ALTERNARIA* SPECIES

The genus *Alternaria* stands distinct from other genera by its transversely and longitudinally septate conidia with a distinct beak, which may vary from very short to very long. The conidia are dark brown, obclavate formed in chain from conidiophore. The conidiophore is dark, broader than the vegetative hyphae, bearing geniculations marked with scars of detached conidia. *Alternaria* belonging to the subdivision Deuteromycotina, class Hyphomycetes and order Moniliales. Based on the conidial ontogeny Subramanian (1965) recognized five distinct families in Hyphomycetes and among them *Alternaria* belongs to the family Helminthosporiaceae. According to the latest classification of fungi (Agrios, 1997) *Alternaria* is classified in Deuteromycetes of class Ascomycetes of Phylum Ascomycota. Perusal of literature revealed many reports on the cultural and morphological characters of *Alternaria* spp. isolated from infected plants.

Ellis (1971) described the morphological characters of different species of *Alternaria*. According to him *Alternaria alternata* (Fr.) Keissler produce black or olivaceous black or sometimes grey colony, conidiophore singly or in groups, simple or branched, straight or flexuous, sometimes geniculate, pale to mid olivaceous or golden brown, smooth, upto 50μ long, 3-6μ thick with one or several conidial scars, conidia formed in long, often branched chains, obpyriform, ovoid or ellipsoidal often with a short conical or cylindrical beak, smooth or verruculose, upto 8 transverse septa and several longitudinal septa, overall length 20-63 (37)μ, 9-18(13)μ thick in the broadest part, beak pale, 2-5μ thick. He also studied the characters of *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire and described as, conidiophores produced solitary or in groups, simple or branched, straight or flexuous, more or less cylindrical, septate, pale, or mid pale brown, smooth, with one or several scars, upto 115μ long, 4-6μ thick, conidia solitary or in short chains, straight or curved, obclavate or with the body of the conidium ellipsoidal tapering gradually to the beak which is upto half the length of the conidium, usually shorter, sometimes tapered to a point but more frequently swollen at the apex where there may be several scars, pale to clear golden brown, usually smooth, sometimes minutely verruculose, generally with 4-7 transverse and several longitudinal septa, overall length 22-95 (54)μ, 8-19 (13.8)μ thick in the broadest part, beak 2-4 μ thick, swollen apex 4-5μ wide.

Subramanian (1971) also described the morphological characters of several species of *Alternaria*. He studied the characters of *Alternaria tenuis* Auct. and described as, conidiophores aggregated into tufts or evenly distributed over the colony. They are simple or branched, erect, olive- brown, septate (septa 5-20 μ apart), variable in length (5-125μ), 3-6μ wide, geniculate, often with several scars and swelling terminally.

Conidia light olive- brown to dark brown, smooth, or warty, with 3-5 transverse septa and with longitudinal septa in the second and third cells, variable in shape, ranging from beakless and ellipsoid, oval or bean shaped to distinctly beaked ones, but typically obclavate with short beaks and borne in long chains, (16-) 20-50 (-70) x (7-) 10-16 (-20) μ. He also described the characters of *Alternaria tenuissima* (Fries) Wiltshire as, hyphae hyaline to olive-buff, septate, 1-6μ wide. Conidiophores olive-buff to dark olive-buff, septate, with nearly 10μ between septa, 20-100 μ long, 3-4.5μ wide, usually simple, erect with 1-6 scars, on the host single or fasciculate, on agar media appearing as single side branches on the hyphae. Conidia in natural media formed in short chains of 2-4, smooth, obclavate, elongate-oval, tapering gradually to a beak, deep to dark olive-buff to buffy brown, 13.5-60 x 6-16.5 μ on natural media, with 2-11 transverse septa, 0-10 longitudinal or oblique septa, slightly constricted at the septa, beak 1.5-45μ long, 2-4.5 μ wide, sometimes swollen terminally, sometimes with 2 or 3 scars, and with 0-8 transverse septa, beak up to the same length as the spore body. Total length of spores 15-105 μ. Conidia forming longer chains on agar media than on natural media, 3-12 (more often 7-10) conidia in each chain.

Neergard (1945) studied the morphological variations of *Alternaria alternata* causing leaf blight of brinjal and reported 7-10 conidia in each chain and 1-9 horizontal and 0-6 longitudinal septa in conidia. Variation in conidial size of *Alternaria alternata* was also reported by Kapoor and Hingorani (1958).

Venkatakrishniah (1953) working with *Alternaria* sp. on *Amaranthus paniculatus* found that the fungus grew well on onion, potato dextrose and oat meal agar media, produced concentric zones and turned dark green to black with age. The sporulation was noted to be more profuse on onion agar than on any of the other media used. Rangaswami and Sambandam (1961) in the study of eight species of *Alternaria* on solanaceous hosts, also obtained maximum growth of all the isolates on potato dextrose agar medium, but on oat meal agar, Czapek's agar, and Richard's agar, the growth was fair and least growth was obtained on nutrient agar.

Varma (1967) studied the cultural characters of seven isolates of *Alternaria* *viz.*, *Alternaria sesamicola*, *Alternaria gomphrenae*, *Alternaria tenuis* and *Alternaria* species from shoe flower, bhindi, basella and onion in five different media. He found that the growth of all the seven isolates of *Alternaria* were generally rapid in different solid media tried even though they took six to ten days to complete the growth in Petri dishes. The mycelial growth of all the seven isolates grown on their respective leaf extract agar was found to be sparse hyaline or greyish hue, while in all the other media tested, these isolates produced profuse aerial mycelium which were variously coloured.

Bhargava and Singh (1985) reported that growth of *Alternaria cucumerina* isolated from cucurbits in malt extract medium and reported that the colonies were circular, cottony, smooth, olivaceous green with definite concentric zonations. They also noted morphological characters of pathogen causing *Alternaria* leaf blight of cucurbits and observed a spore length ranged between 36-108 µm, beak length 36- 41µ m with 1-4 cross septa and 1-3 longitudinal septa.

Pandey and Vishwakarma (1999) studied the morphological and symptomatological variations in *Alternaria alternata* causing leaf blight in brinjal. They observed 4-8 conidia in chain formed in acropetal manner on short conidiophore. The size of conidia from pure culture varied from 13.3-37.2 µm in length and 6.7-13.3 µm in width, often beak of an average 3.4µm long.

Babu *et al.* (2000) reported variations in conidial morphology of *Alternaria solani* causing leaf blight of tomato. Conidial morphology and development of *Alternaria cassiae* from cowpea was studied by van-den- Berg *et al.* (2002). They reported that conidia of *Alternaria cassiae* were large, obclavate and formed singly or in chains and the conidiophores were straight or curved and enlarged apically at the site of conidium production. They observed smooth, bud like conidial initials at the apex of conidiophores. As conidia matured, they became elliptical to obovate and densely verrucose.

The conidial morphology of *Alternaria solani* isolated from tomato was studied by Ahamad (2002) and found that the conidial length varied from 175.6-270.5 µm and the width ranged from 12.5-16.5 µm with 5-11 septa. The isolate produced beaked and unbeaked conidia. The beak was flexuous, brown and sometimes branched. The beak was 47.0- 65.5µm long and 2.5-5µm wide, tapering gradually.

Cultural and morphological characters of *Alternaria alternata* from ivy gourd were studied by Davis (2003) and reported that the fungus took only seven days to complete full growth in 9cm Petri dishes. The growth initially showed whitish colour, later lower portion of the colony turned dark brownish black colour. The mycelial growth had a velvety appearance with dark purplish tinge on the upper side and reverse of the colony was almost black. She identified *Alternaria alternata* as the causal organism of leaf spot in ivy gourd and described the morphological characters of the conidia as spore length ranged between 19.5-31.2µm, width between 7.8-15.6µm, 3-5 transverse septa and 1-3 longitudinal septa.

2.4 PHYSIOLOGICAL AND NUTRITIONAL CHARACTERS ON GROWTH AND SPORULATION OF *ALTERNARIA* SPECIES

Newton (1946) and Srivastava (1951) reported good growth of various species of *Alternaria* on medium containing potassium nitrate as nitrogen source. In general, nitrogen has been reported to be the most favourable for the mycelial growth of many fungi (Lilly and Barnett, 1951). They stated that the majority of the fungi grew satisfactorily between p^H 5.0 and 6.0. They also reported that lactose is utilized by far fewer fungi than other saccharides. Singh and Prasada (1973) and Baker and Grewal (1987) reported that amongst the nitrates, potassium nitrate and calcium nitrate supported maximum growth of many fungi.

Ansari *et al.* (1989) studied the effect of temperature, RH, p^H and exposure to light and darkness on growth and sporulation of *Alternaria brassicae* causing *Alternaria* blight of rapeseed and mustard. They observed that the growth and sporulation occurred at 5-30°C and were optimum at 23°C. Mycelial growth reached a maximum at p^H 6.5.

Karlatti and Hiremath (1989) studied the conditions for maximum conidial germination of *Alternaria zinniae* and observed a good germination of conidia in water but they got maximum germination at a temperature range of 25-30°C (opt .25°C), p^H 5-5.6 (opt.5.3) and RH >90%.The factors affecting growth and sporulation of *Alternaria brassicola* was studied by Kundu *et al.* (1991). They found that germination was high at high temperature, neutral p^H, and over a temperature range of 25-35 °C and it remained cent per cent irrespective of intensity and quality of light.

Choulwar and Datar (1991) studied the effect of temperature and light on growth of *Alternaria solani* causing early blight of tomato. They found that the optimum temperature for growth on potato dextrose agar was 28°C. Exposure to sunlight produced maximum growth and distinct zonations but not sporulation. Later Ayub *et al.* (1995) studied the effect of temperature, p^H, light , carbon and nitrogen sources on growth of *Alternaria cyamopsidis* causing leaf blight of guar (*Cyamopsis tetragonaloba*). Potato dextrose agar was found to be the best medium for growth and sporulation. Growth on artificial medium was best at 30 ± 2°C, p^H 6, with glucose as carbon source, KNO_3 as nitrogen source and 12h cycles of light and darkness. But Saeed *et al.* (1995) reported Richard's agar medium as best medium for the growth of *Alternaria* and maximum colony growth at 27°C and p^H 5.5. Glucose and potassium nitrate were reported to be the best carbon and nitrogen sources, respectively for maximum mycelial growth.

The effect of culture media, temperature, p^H and inorganic sources of nitrogen on growth and sporulation of *Alternaria brassicola*, isolated from *Alternaria* blight infected cabbage leaves, was studied by Khatun *et al.* (1996). They observed that host leaf extracts and potato dextrose agar media supported growth and sporulation of the fungus. Mycelial growth was maximum within the range of 20-30°C and p^H 6-8 with maximum growth occurring at 25°C and p^H 7.0. Of the nitrogen sources tested, urea and KNO_3 had a positive effect on the growth and sporulation of the fungus.

Benlioglu and Delen (1996) examined nine different artificial media in combination with four different light and temperature conditions along with several other media for their ability to induce sporulation of *Alternaria solani* from tomato in *in vitro* condition. T media containing tomato juice was found to be the most suitable with 6 days dark at 23 °C, 12 h light at 26° C and 12 h dark at 18° conditions for sporulation of different isolates of *Alternaria solani.*

Srinivas *et al.* (1997) reported that sunflower leaf agar with 200g of sunflower leaves per liter of medium without dextrose was found to be an excellent medium for mass multiplication of *Alternaria helianthi* causing leaf blight of sunflower followed by oat meal agar and Czapek's agar media.

The effects of culture media, temperature, p^H and inorganic sources of nitrogen on the growth and sporulation of *Alternaria porri* isolated from purple blotch infected onion leaves from Bangladesh were studied by Hossain *et al.* (1997). Onion leaf extract and potato dextrose agar were found to be the media supported the growth and sporulation of the fungus. Luxuriant mycelial growth was obtained within a temperature range of 20-30°C and p^H 6-8 having maximum growth at 25°C and p^H 7.0. Among the sources of nitrogen, KNO_3 and urea positively influenced the growth and sporulation of the fungus. Thind (1997) reported fair growth of *Alternaria alternata* on lactose. But on the contrary, lactose proved to be the poorest carbon source for the growth of various fungi (Sahni *et al.*, 1975., Garcha and Singh, 1976., Anilkumar and Sastry, 1980).

Physiological studies on *Alternaria alternata* (Fr.) Keissler a causal agent of leaf blight of turmeric was done by Gaddankeri and Kulkarni (1998). They found that the fungus achieved maximum growth at 25°C on the twelfth day of incubation beyond which growth declined. Among the different liquid media, Richard's medium supported maximum mycelial growth. Fungal growth was greatest at p^H 6.5 and at 25°C. Kumari *et al.* (1998) also reported that, Richard's medium both in its solid and liquid state as best for supporting maximum mycelial growth and sporulation of *Alternaria brassicae*. They recorded the temperature of 25°C and p^H 5.5 as the optimum for growth and sporulation of the pathogen. Amongst the different media tested, they found that one per cent sucrose solution supported maximum conidial germination followed by one per cent glucose solution and leaf extract of cauliflower.

Maheshwari *et al.* (2000a) recorded that the optimum temperature and p^H for the growth of *Alternaria alternata* causing leaf spot of dolichos bean were 28°C and p^H 6.5. They also observed that minimum fungus growth was at 5°C and at p^H 10.5 and excellent sporulation at 25-30°C and p^H 5.5-6.5.Effect of temperature, light/ darkness and RH on germination and sporulation of *Alternaria tenuissima* was studied by Singh *et al.* (2001a). They found maximum germination at 25°C, in total darkness and 100% RH, maximum germ tube length was at 25°C, in total darkness and 90%RH, maximum number of germ tubes and conidium was at 25°C and 100%RH and maximum number of spores in chain at total darkness and 90%RH.

The germination characteristics of conidia of *Alternaria tenuis* was studied by Chen *et al.* (2000). They found that spores could germinate at a temperature of 8-36 °C, 33-100% RH, in fluid pseudo ginseng plant extracts, 1% glucose, and under 24h darkness and 24 h light. However optimum conditions were a temperature of 18-25°C, 90-100%RH, use of pseudo ginseng plant extract, and 12h darkness and 12h light. Influence of different media, p^H and temperature on growth and sporulation of *Alternaria alternata* (Fr.) Keissler, causing *Alternaria* blight of Chickpea was studied by Singh *et al.* (2001b). They found that among different media, maximum colony size was obtained on potato dextrose agar and the optimum temperature for growth and sporulation was at 25°C and the optimum p^H was 5.5.

Hait (2002) studied the effect of different media on growth and sporulation of *Alternaria alternata* pathogenic to *Solanum khasianum*. He found that among the 10 media tested, solid and liquid media of potato dextrose agar showed the best growth and sporulation, which were significantly on par with oat meal agar medium. Maximum sporulation occurred in both solid and liquid media of potato dextrose, oat meal and malt extract but comparatively less mycelial growth was observed in malt extract. The poorest growth with the least sporulation was observed in Conn's medium.

Influence of temperature and light on the growth and *in vitro* sporulation of *Alternaria helianthi* was studied by Raranciuc (2002). He observed that the sporulation was greatest at 20-26°C, while conidial germination decreased with increasing temperatures above 25°C, and was also reduced at temperatures below 20°C. They found that light had a positive influence, resulting in more rapid growth and abundant fructification of the fungus.

Jash *et al.* (2003) studied the effect of different physiological factors on growth and sporulation of *Alternaria zinniae* causing leaf and flower blight of marigold. They found that maximum mycelial growth was observed on liquid and solid leaf extract dextrose media. Sporulation was most pronounced on the liquid and solid forms of leaf extract dextrose and malt extract. *Alternaria zinniae* was able to grow at p^H of 5 to 8.5, but optimum growth was recorded at p^H 6 to 6.5. Among the carbon sources, sucrose resulted in the highest average mycelial dry weight. Sporulation was greatest in the medium containing sucrose, starch and mannitol.

Davis (2003) studied the spore germination characteristics of *Alternaria alternata* on ivy gourd. She found that the species took three hours to start germination and ten hours for attaining cent per cent germination. She also observed that all the cells of the conidium were germinated to produce germ tube which later elongated to form hyphae.

Saharan *et al.* (2003) studied the spore germination of *Alternaria cucumerina* var. *cyamopsidis* causing *Alternaria* blight of cluster bean at different temperatures. They observed that the conidial germination initiated 4h of incubation at all temperatures (20, 24, 28 and 32°C). The maximum germination of conidia was observed at all temperatures after 14h of incubation.

2.5 HISTOPATHOLOGICAL STUDIES

Munjal and Gupta (1965) observed during the study of parasite relations of the anthracnose of celosia that the cells of diseased tissue lost their shape and later the hyphae collected underneath the epidermis from stroma, from which conidiophores were produced.

Tebust *et al.* (1978) reported that when the weed plant aeschynomene was infected with *Colletotrichum gloeosporioides* the mycelium grew within the cortex cambium, xylem and pithway tissues and death of seedlings occurred.

Hyphal structures of *Alternaria zinniae* were observed in the cross sections of inflorescences of marigold (*Calendula officinalis*) by Karlatti and Hiremath (1989). They noticed that the infection leads to disorganization of epidermal and parenchyma tissues of petal, involucre of bracts, ray and disc of florets. Destruction of pollen grains was evident.

The histology and development of *Alternaria helianthi* in sunflower leaf tissues were studied by Thaiz and Subero (1997) in whole mounts, and transverse and longitudinal sections of inoculated areas of leaves. Observations indicated that a minute infection peg was produced on the under side of the appressorium which entered the epidermal cell and formed primary hyphae that grew intracellularly. Later, secondary hyphae developed from the primary hyphae, grew intracellularly between the palisade cells and branched intercellularly in all directions to the other tissues. Histological alterations induced by the fungus were observed in the epidermal, mesophyll and vascular tissues, which showed cellular collapse and necrosis. In addition, infected leaves showed reduced leaf thickness.

Histopathology of infection of *Minneola tangelo* by *Alternaria alternata* pv. *citri* was studied by Solel and Kimchi (1998). They found that after inoculation of *Minneola tangelo* leaves with conidia of *Alternaria alternata* pv. *citri*, the germ tubes randomly grew over the leaf surfaces and twenty four hour after inoculation, appressoria appeared over the epidermal cells, they formed infection hyphae that penetrated in to the leaf, and developed intercellular branching hyphae which resulted in minute necrotic lesions. The lesions grew in to large necrotic spots with in 2-3 days, subsequently accompanied by a dark discoloration of the veins extending from the lesions.

Gomez *et al.* (2001) studied the histopathology of *Alternaria solani* on tomato associated with marigold (*Tagetes erecta* L.) and Pigweed (*Amaranthus hypochondriacus*). Samples of foliar tissues were taken 4, 12, 24 and 48 hour after inoculation, to determine the percentage of germinated conidia, number, length and orientation of germ tubes per conidium and to studied the penetration and colonization by the fungus.

Histopathological changes due to *Alternaria* infection on ivy gourd was observed by Davis (2003). She noticed the destruction of sub epidermal cells and disintegration of chlorophyll grains. In the advanced stages, necrosis and complete death of cells were noticed.

2.6 HOST RANGE

Kapoor and Hingorani (1958) found that *Alternaria tenuis* from brinjal (*Solanum melongenae*) infected potato, tomato and *Hyoscyamus niger*. Berry (1960) observed that *Alternaria sesami* was non- pathogenic to other crop plants inoculated, and *Alternaria solani* from tomato and potato and *Alternaria cucumerina* from cucurbits did not attack sesame.

Khandelwal and Prasada (1970) during their host range study reported that *Alternaria* sp. isolated from watermelon was found to produce infection on muskmelon and cucumber.

Host range of *Alternaria alternata* f.sp. *cucurbitae* causing leaf spot of cucumber was studied by Vakalounakis (1990b). A total of 62 cultivated and weed species in 16 families were artificially inoculated or exposed to natural infection in green house experiments. Of these, 27 species of cucurbitaceae were found to be susceptible to a forma specialis of *Alternaria alternata* that attacks cucumber. To differentiate this *Alternaria alternata* from others it was designated *Alternaria alternata* f.sp. *cucurbitae.*

Ansari *et al.* (1990) studied the host range of *Alternaria brassicae*. 50 taxa belonging to 15 families of various crops were inoculated with *Alternaria brassicae*. All cruciferae were infected, but only 3 species from the other families (*Chenopodium album, Convolvulus arvensuis* and *Anagallis arvensis)* became infected.

The host range of *Alternaria alternata* f.sp. *sphenocleae* causing leaf blight of *Sphenoclea zeylanica* was studied by Masangkay *et al.* (1999). Forty eight plant species in 40 genera representing 20 families were screened for susceptibility to *Alternaria alternata* f.sp. *Sphenocleae* and found that goose weed (*Sphenoclea zeylanica*) was the only species susceptible to *Alternaria alternata* f .sp. *sphenocleae.*

The host range of *Alternaria cirsinoxia*, the effect of temperature on mycelial growth and survival and over wintering on Canada thistle were studied by Green *et al.* (2001). They found that *Alternaria cirsinoxia* was pathogenic to all the asteraceae species tested with the highest disease rating being recorded on Safflower, sunflower *C. arvense*, aster and *Centurea diffusa.*

2.7 EFFECT OF TOXIN ON DISEASE DEVELOPMENT

Kodama (1991) reported the production of AL toxin by *Alternaria alternata* tomato pathotype. Culture filtrates of several *Alternaria solani* isolates were tested for their phytotoxicity to tomato genotypes previously evaluated for resistance to early blight and collar rot by Maiero *et al.* (1991). Toxins produced by *Alternaria* species and their biosynthesis and toxicology are discussed by Coulombe *et al.* (1991).

The physical and chemical properties, analytical methods, toxicity and natural occurrence of some major *Alternaria* toxins including alternariols and altenuenes, altertoxins, tenuazonic acid and AAL toxins, are studied by Visconti *et al.* (1994). Variability in *Alternaria brassicae* in response to toxin production was studied by Vishwanath and Kolte (1997) on cruciferous hosts and found that among the three isolates tested the toxin extracted from one isolate of the pathogen produced typical symptom on detached leaf. Two phytotoxins were isolated from culture filtrates of an *Alternaria alternata* isolate which was pathogenic to sunflower by Liakopoulou *et al.* (1997). One of the toxins was identified by chemical and physicochemical techniques and named as AS-I toxin which was further characterised by its phytotoxic effect on sunflower and other plants.

Otani *et al.* (1998) studied the production of a host specific toxin by germinating spores of *Alternaria brassicola*. In laboratory studies, spore suspensions of *Alternaria brassicola* pathogenic to brassica species were incubated on the surface of brassica leaves. Fluid produced by germinating spores was collected and examined for biological activity. Using a detached brassica leaf bioassay, spore fluid induced water soaking symptoms followed by brown necrotic lesions in susceptible plants, but did not cause visible symptoms in nonhost leaves.

The effect of culture filtrates of *Alternaria alternata* on leaves of dolichos bean (*Lablab purpureus*), seed germination and seedling vigour was evaluated *in vitro* by Maheshwari *et al.* (2000b). They found that all the leaves showed symptoms of *Alternaria* leaf spot after application of the culture filtrate. Seed germination and seedling growth (root and shoot length) was inhibited by unheated culture filtrate, at a concentration of 1:10 dilution.

Mesbah *et al.* (2000) tested the AAL toxin produced by *Alternaria alternata* f. sp. *lycopersici* on 200 species of solanaceae and recorded 25 species sensitive to the toxin at a concentration of 0.2μm. The effect of exo and endo toxins of *Alternaria alternata* in disease development on ivy gourd was studied by Davis (2003). She observed slight yellow discolouration on endotoxin inoculated leaves and no symptoms were observed on leaves inoculated with exotoxin.

Singh (2003) reported the production of nonhost specific toxin by *A. alternata* and development of striking variegated chlorosis in cucumber, cotton, citrus and other plants by both toxin and pathogen.

Materials and Methods

3. MATERIALS AND METHODS

The present study on 'Variability of *Alternaria* isolates causing leaf blight diseases in cucurbits" was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during the period from January 2004 to August 2005. The details of the materials used and the techniques adopted for the investigation are described below.

3.1 SURVEY AND COLLECTION OF DISEASE SAMPLES

A survey was conducted in the vegetable fields of Department of Olericulture, College of Horticulture, Vellanikkara and in the farmer's fields at Elanad, Thrissur district to study the occurrence of leaf blight diseases of cucurbits. Disease samples were collected periodically from all cucurbitaceous plants *viz.*, gourds, melons, cucumbers and pumpkins whichever were available in the field at the time of survey.

3.2 ISOLATION AND MAINTENANCE OF *ALTERNARIA* SPECIES ASSOCIATED WITH LEAF BLIGHT DISEASES IN CUCURBITS

Leaves showing fresh lesions were collected, brought to the laboratory, washed under tap water and were dried with blotting paper. Specimens were then kept under moisture chamber for stimulating sporulation and observed under stereomicroscope for the presence of spores of *Alternaria*. Simultaneously small bits of infected leaf portions along with some healthy areas were surface sterilized with one per cent sodium hypochlorite, then washed in three changes of sterile water and were transferred to Potato Dextrose Agar (PDA) medium. The fungus grown on the medium was purified by single spore isolation technique and the isolates were periodically subcultured and maintained on PDA medium for further investigations.

3.3 PATHOGENICITY

The pathogenicity of *Alternaria* isolates associated with leaf blight disease of various cucurbits was studied by artificial inoculation in respective hosts under *in vitro* and *in vivo* conditions.

3.3.1 *In vitro* condition

Healthy leaves of various cucurbits were collected from the field, washed under tap water and then disinfected with 70 per cent ethyl alcohol. The leaves were inoculated separately with 10 mm mycelial disc of the isolates on both surfaces with or without pinpricks. Leaves inoculated with sterile water served as control. The inoculated leaves were kept in humid chamber and observed daily for the symptom expression. The pathogen was reisolated from the inoculated leaves showing the typical symptom and compared with the original culture (Plate 2a).

3.3.2 *In vivo* condition

Cucurbitaceous vegetables *viz.,* gourds, melons, cucumbers and pumpkins were grown in polybags. At flowering stage, five middle aged leaves were inoculated separately on both surfaces with 10 mm mycelial disc of the isolate with and without pinpricks. Plants inoculated with sterile water served as control. The leaves were covered with polythene covers for 48h after inoculation and observed for the symptom expression. The pathogen was reisolated from the inoculated plants showing symptoms and compared with the original culture (Plate 2b).

3.4 SYMPTOMATOLOGY OF THE DISEASE

Symptoms developed on the leaves of different cucurbits by the respective isolates of *Alternaria* were studied in detail both under natural and artificial conditions. The shape, size and colour of the infected area from the initiation of infection to the final stage of development of disease were observed in the natural condition. To study the symptom development in artificial condition, the different isolates were inoculated on the detached leaves of its respective hosts as described earlier. The symptoms developed on the leaves were compared with that in natural condition.

3.5 IDENTIFICATION AND STUDY ON VARIABILITY OF ISOLATES OF *ALTERNARIA*

The cultural, morphological, physiological and nutritional characters of various isolates of *Alternaria* sp. associated with leaf blight disease in different cucurbitaceous vegetables were studied in detail for their identification and also to study any variability existing among them.

3.5.1 Cultural characters of different isolates of *Alternaria*

Cultural characters of the different isolates of *Alternaria* such as colour, shape and texture of the colony, sporulation and growth rate in the medium were studied in detail and observations on colony colour, appearance of aerial mycelium and development of concentric zonations in PDA medium were recorded.

Spore count of the different isolates of *Alternaria* was taken using haemocytometer. Spore suspension was prepared in five ml of sterilized water by transferring 10 mm diameter disc from the 10 day old culture of the isolates. The suspension was shaken thoroughly to disburse the spores in to water. The spore count per ml of spore suspension was calculated by using haemocytometer. The formula is given below,

$$\text{Number of spores/ ml} = \frac{X \times 400 \times 10 \times 1000 \times D}{Y}$$

Where

X = Total number of spores counted

Y = Number of smaller squares checked

10 = Depth factor

D = Dilution factor

1000 = Conversion factor for mm^3 to cm^3

To study the growth rate of different isolates of *Alternaria* PDA, Host leaf extract agar medium, Richard's agar medium, Czapek (Dox) agar medium and Selective medium for *Alternaria* were used. For this, different types of media were prepared and transferred aseptically into sterile Petri dishes. Inoculation of the different isolates was done by centrally placing circular mycelial discs of 10 mm diameter, taken with a cork borer from actively growing 10 day old culture of the isolates. Three replications were kept for each isolate in different media. The plates were incubated at room temperature. The growth rate of different isolates of *Alternaria* was recorded by measuring the diameter of the growth of culture from second day after inoculation till complete the full growth on Petri dishes. The composition of different media used is given in Appendix I.

3.5.2 Morphological characters of different isolates of *Alternaria*

Morphological characters of different isolates of *Alternaria* in pure cultures were studied. Permanent slides were prepared from the pure culture of all the isolates. Using micrometer, measurements on the size of hyphae, length and breadth of conidia, length and breadth of conidiophores and the number of transverse and longitudinal septa of the conidia of all the isolates were taken. Measurements on 50 spores of each isolate were recorded. Camera lucida drawings and photomicrographs of conidia and conidiophores were made. These characters were compared with the characters given in CMI descriptions of fungi and bacteria and in text book "Dematiaceous Hyphomycetes" (Ellis, 1971) to identify the species of *Alternaria.*

The morphological characters of different *Alternaria* isolates were analyzed with Euclidean co-efficient and was clustered by the Unweighed Pair Group Average Method (UPGMA: Sneath and Sokal, 1973) using NTSYS pc 2.02 software to produce grouping. The genetic dissimilarity matrix was also computed.

3.5.3 Physiological and nutritional characters of different isolates of *Alternaria*

Richard's broth was used to evaluate the effects of various physiological and nutritional factors *viz.*, temperature, p^H, light, carbon and nitrogen sources on the growth of different isolates of *Alternaria.*

3.5.3.1 Effect of temperature on the growth of different isolates of Alternaria

Effect of temperature on the growth of different isolates of *Alternaria* was tested at 25°, 30° and 35°C. Using a cork borer 10 mm diameter disc of 10 day old fungal growth was cut and transferred to conical flasks containing 100 ml Richard's broth and incubated at three temperatures in a BOD incubator. Three replications were kept for each isolate. When the fungal growth in any one of the treatment completely cover the surface of liquid medium, the broth cultures were filtered separately through Watman No.1 filter paper and took the fresh weight of the mycelium after deducting the weight of the filter paper. Mycelial growth of all the isolates were oven dried at 50°C and the dry weight was taken every day till three consecutive constant weight was obtained.

3.5.3.2 Effect of p^H on the growth of different isolates of Alternaria

Different p^H tested for the growth of the fungus was 6, 7 and 8. P^H of Richard's broth was adjusted to acidic and alkaline by using dilute hydrochloric acid and sodium hydroxide solutions respectively. p^H paper was used for testing the p^H of the medium. Three replications were kept for each p^H. Fungal discs of 10 mm diameter were transferred to the 100 ml liquid medium and incubated at room temperatures. The fresh weight and dry weight of the mycelia were taken in the same manner as explained above.

3.5.3.3 Effect of light on the growth of different isolates of Alternaria

Conical flasks containing 100ml Richard's broth was inoculated with 10 mm disc of 10 day old fungal growth. Three replications were kept for each isolate. The inoculated flasks were exposed to full light, alternate light and darkness and full darkness. Broth cultures were exposed to fluorescent lamp in night to get full light. For providing full darkness broth cultures were covered with black paper. The fresh weight and dry weight of the fungal growth were recorded as in the same way as explained in 3.5.3.1.

3.5.3.4 Effect of carbon sources on the growth of different isolates of Alternaria

Different carbon sources selected for the study were sucrose, starch and lactose. Hundred millilitre Richard's broth with above mentioned carbon sources were inoculated separately with 10 mm disc of 10 day old culture of different isolates of *Alternaria* and incubated at room temperature. Observations on fresh weight and dry weight of the isolates were taken in the same manner as explained in 3.5.3.1.

3.5.3.5 Effect of nitrogen sources on the growth of different isolates of Alternaria

Different nitrogen sources tested to evaluate the growth of the fungus were KNO_3, $MgNO_3$ and $NaNO_3$. Using a cork borer 10 mm disc of 10 day old culture of different isolates of *Alternaria* were cut and inoculated separately to flasks containing 100 ml Richard's broth and incubated at room temperature. Three replications were kept for each isolate. Observations on the fresh weight and dry weight of the fungal growth were taken as explained in 3.5.3.1.

3.6 CONIDIAL GERMINATION OF DIFFERENT ISOLATES OF *ALTERNARIA*

Spore suspension of the different isolates were prepared separately by transferring 10 mm disc of fungal growth from pure culture to five ml sterilized water and then filtered through muslin cloth to remove mycelial bits. Transferred few drops of the spore suspension to a sterilized, clean cavity slide using a micropipette and covered with cover slip. The slides were observed under the microscope and noted the number of spores in a microscopic field. The slides were then kept inside a humid chamber for germination of spores. Observation on number of spores germinated was taken at hourly interval for a period of maximum conidial germination. The germination percentage of spores was calculated using the formula given below.

$$\text{Per cent germination} = \frac{\text{Number of spores germinated}}{\text{Total number of spores}} \times 100$$

3.7 COMPATIBILITY BETWEEN DIFFERENT ISOLATES OF *ALTERNARIA*

To study the compatibility between different isolates of *Alternaria*, the dual culture technique of Dennis and Webster (1971) was used. In 90 mm sterile Petri dishes, sterilized plain agar medium was poured and allowed to solidify and with the help of two forceps sterilized cellophane discs of 90 mm diameter was placed over this so as to lie flat on the surface of the medium. Agar discs of 10 mm diameter with actively growing mycelium of *Alternaria* isolated from two different hosts of the same location were placed at two centimeter apart on the cellophane paper and were incubated at room temperature. Microscopic observation on hyphal interaction was made by cutting out one sq.cm portion of cellophane paper containing intermingling hyphal growth of the different isolates and mounting in cotton blue stain. Similarly compatibility of all the isolates of the same and different locations was studied.

3.8 HISTOPATHOLOGICAL STUDIES

Thin sections of leaves of cucurbitaceous vegetables infected with isolates of *Alternaria* were taken and stained with Saffranin and observed under microscope to study the histopathological changes brought about by the the pathogen. These were then compared with the sections made from healthy leaves.

3.9 HOST RANGE

Host range study was conducted by cross inoculating the different isolates of *Alternaria* on different cucurbitaceous vegetables and also on other vegetables *viz.*, tomato, chilli, brinjal, amaranthus, cowpea and bhindi. For that all crops were raised separately in polythene bags filled with potting mixture. Cross inoculation with various isolates of *Alternaria* were done on both surfaces of lower four matured leaves with and without giving pinpricks. The inoculated leaves were covered with polythene bags to provide sufficient humidity for infection and symptom development on leaves was noticed from next day onwards. The pathogen was reisolated from the inoculated leaves and pure cultures were compared with the original culture obtained from its host.

3.10 EFFECT OF TOXIN ON DISEASE DEVELOPMENT

An *in vitro* experiment was conducted to evaluate the effect of toxins extracted from various isolates of *Alternaria* on the development of leaf blight symptoms on the respective hosts of the isolates and also on other vegetable *viz*., tomato, chilli, brinjal, amaranthus, cowpea and bhindi.

Hundred millilitre of Richard's liquid medium was sterilized in 250 ml conical flask and inoculated separately with mycelial discs of 10 mm diameter obtained from the actively growing 10 day old culture of each isolate. These flasks were incubated at room temperature till the fungal growth fully covered the surface of the broth. Then the broth culture was filtered through sterile Watman No.1 filter paper to extract exotoxin. Fresh weight of the mycelial mat was taken and homogenized separately with five times sterilized water and centrifuged at 1000 rpm. for 15 min. Pellets were discarded and the supernatant solution was again centrifuged for 15 min. at 1000 rpm. The supernatant after second centrifugation was taken as endotoxin.

Plants were raised in polybags with two plants in each bag. Matured leaves were inoculated separately with exotoxin and endotoxin on both surfaces of the leaves after giving injury. The inoculated plants were covered with polythene cover to maintain humidity and observed daily for the development of symptoms. Plants inoculated with sterile water served as control.

3.11 STATISTICAL ANALYSIS

Analysis of variance was performed on the data using the statistical package **MSTATC** (Freed, 1986). Multiple comparisons among treatment means were done using **DMRT.**

Results

4. RESULTS

The present investigation was carried out to find out whether any variability existing among the isolates of *Alternaria* causing leaf blight in cucurbits. For this studies were carried out on the symptomatology, cultural, morphological, physiological and nutritional characters of the different isolates, host range and effect of toxic metabolites of the isolates in symptom expression and the results are presented below.

4.1 ISOLATION AND MAINTENANCE OF VARIOUS ISOLATES OF *ALTERNARIA* ASSOCIATED WITH LEAF BLIGHT DISEASE IN CUCURBITS

Isolation of *Alternaria* from disease samples of ash gourd, snake gourd, ridge gourd, bottle gourd, bitter gourd, ivy gourd and pumpkin collected from Vellanikkara and Elanad areas of Thrissur district was done in PDA. Seven isolates of *Alternaria* were obtained from Vellanikkara and six isolates from Elanad area. *Alternaria* isolate of pumpkin from Elanad area was not obtained. All the 13 isolates were brought into pure culture on PDA medium and sub cultured at frequent intervals.

4.2 PATHOGENICITY

Pathogenicity of different isolates of *Alternaria* associated with leaf blight diseases of cucurbitaceous vegetables were proved by artificial inoculation under *in vitro* and *in vivo* conditions in their respective hosts.

4.2.1 *In vitro* condition

On artificial inoculation the isolates of *Alternaria* on detached leaves of their respective hosts produced the symptom within two to four days. Reisolation of the pathogen from the inoculated leaves yielded colonies similar to that of original culture.

4.2.2 *In vivo* condition

In *in vivo* condition, the leaves of cucurbitaceous vegetables in polybags inoculated with its respective isolates developed the typical symptoms within five to seven days. Pathogens reisolated from inoculated portions were similar to the colonies of original culture.

4.3 SYMPTOMATOLOGY

Symptomatology of *Alternaria* leaf blight disease observed in ash gourd, snake gourd, ridge gourd, bottle gourd, bitter gourd, ivy gourd and pumpkin were studied in detail under natural and artificial conditions. A slight variation in symptom expression in different host plants was noticed. But the symptoms observed at two different locations were similar.

4.3.1 Symptoms on cucurbitaceous vegetables under natural condition

4.3.1.1 Ash gourd

Symptoms developed from the margins of leaves as small brown irregular lesions with clear yellow halo on both surfaces of the leaves. Later the lesions turned to dark brown and papery with characteristic zonations. The size of lesions ranged from 0.3 to 2.5 cm. In severe cases such lesions coalesced together resulting in leaf blight symptoms. Older leaves were found more susceptible to *Alternaria* infection (Plate 3a).

4.3.1.2 Snake gourd

Generally the symptoms were seen on older leaves. It developed as small light brown irregular lesions from leaf tip and margins. The lesions were surrounded by an yellow halo. As the disease advanced towards the midrib, lesions coalesced and became larger, irregular, turned dark brown papery blightened area. No clear zonations were observed in the infected areas. The size of lesions ranged from 0.2 to 3.5 cm (Plate 3b).

4.3.1.3 Ridge gourd

On older leaves, symptoms developed as small, brown, irregular lesions with characteristic yellow halo from the margins of leaves. Later these lesions coalesced and became dark brown on colour with papery texture. Circular zonations were absent. The size of lesion varies 0.5 to 3.0 cm (Plate 3c).

4.3.1.4 Bottle gourd

Symptoms appeared on older leaves as small, irregular brown lesions with yellow halo from the margins of the leaves. The brown lesions enlarged in size and coalesced. These coalesced lesions covered most of the leaf area and finally results in blightening symptom. Concentric zonations were absent. The size of lesions ranged from 0.5 to 3.0 cm (Plate 3d).

4.3.1.5 Bitter gourd

Symptoms developed first on the older leaves as small, irregular brown lesions from the margins the leaf. Later these brown lesions coalesced and enlarged in size with characteristic yellow halo. Typical concentric zonations were conspicuous. Size of lesions ranged from 0.3 to 2.5 cm (Plate 3e).

4.3.1.6 Ivy gourd

Symptoms started from the margins of the leaves as small, brown, irregular lesions with clear yellow halo on both surfaces of the leaves. Later the lesions turned to dark brown and became papery with characteristic concentric zonations. In severe cases the lesions coalesced resulting in leaf blight symptoms. Older leaves were found more susceptible to infection. The size of lesions ranged from 0.3 to 2.5 cm (Plate 3f).

4.3.1.7 Pumpkin

Symptoms appeared on margins of older leaves as small irregular brown lesions with yellow halo. Later the lesions coalesced together, enlarged in size and

Plate 1. General symptom of *Alternaria* leaf blight in cucurbits

a. Ash gourd

b. Bitter gourd

c. Ivy gourd

Plate 2. View of artificial inoculation of the pathogen

a. *In vitro* condition

b. *In vivo* condition

Plate 3. Symptomatology of *Alternaria* leaf blight diseases of cucurbits

A. Under natural condition

a. Ash gourd

b. Snake gourd

c. Ridge gourd

d. Bottle gourd

e. Bitter gourd

f. Ivy gourd

g. Pumpkin

progressed towards midrib. Finally lesions became dark brown, later dried up and became papery. Concentric zonations were absent. The size of lesions ranged from 0.5 to 3.2 cm (Plate 3g).

4.3.2 Symptoms on cucurbitaceous vegetables under artificial condition

Under artificial inoculation, variations in the symptom expression by the isolates of *Alternaria* on their respective hosts were observed as compared to those noticed under natural condition. Symptoms developed as irregular yellow specks on the inoculated area of the leaves. Later, within two to four days of inoculation, yellow coloured area turned to dark brown in colour with yellow halo. These lesions enlarged in size and covered most of the leaf area. And finally remaining portion of leaf turned yellow with marginal necrosis. The symptom development was found to be similar in all other crops inoculated. It was found that injury by pinprick on lower side of the leaves favoured faster development of symptom. No variations in symptom development were noticed when isolates from two different locations were inoculated (Plate 4a to 4g).

4.4 IDENTIFICATION AND VARIABILITY AMONG DIFFERENT ISOLATES OF *ALTERNARIA*

The cultural, morphological, physiological and nutritional characters of different isolates of *Alternaria* from different cucurbitaceous vegetables were studied to identify them and also to understand whether any variations existed among the isolates.

4.4.1 Cultural characters

Cultural characters of the different isolates of *Alternaria* such as colony characters, sporulation and growth rate were studied in detail and the results are presented in Table 1 to 10.

4.4.1.1 Colony characters of isolates Alternaria

Colour, shape and other colony characters of *Alternaria* were studied and the details are described in Table 1. Various isolates of *Alternaria* produced brownish grey to

Plate 4. Symptomatology of *Alternaria* leaf blight diseases of cucurbits

A. Under artificial condition

a. Ash gourd

b. Snake gourd

c. Ridge gourd

d. Bottle gourd

e. Bitter gourd

f. Ivy gourd

g. Pumpkin

Table 1. Colony characters of different isolates of *Alternaria*

Sl. No.	Isolates	Locations	
		Vellanikkara	Elanad
1	Ash gourd	Aerial mycelium brownish grey, colony thick and velvety, concentric zonations more conspicuous, on the reverse side the colony is black.	Aerial mycelium brownish grey, colony thick and velvety, concentric zonations absent, on the reverse side the colony is black.
2	Snake gourd	Aerial mycelium brownish grey, colony thick and velvety, concentric zonations absent, on the reverse side the colony is black.	Aerial mycelium brown, colony thick and velvety, concentric zonations more conspicuous, on the reverse side the colony is black.
3	Ridge gourd	Aerial mycelium brownish grey, colony thick and velvety, concentric zonations more conspicuous, on the reverse side the colony is black.	Aerial mycelium brownish grey, colony thick and velvety, concentric zonations more conspicuous, on the reverse side the colony is black.
4	Bottle gourd	Aerial mycelium brownish grey, colony thick and velvety, concentric zonations absent, on the reverse side the colony is black.	Aerial mycelium brownish grey, colony thick and velvety, concentric zonations more conspicuous, on the reverse side the colony is black.
5	Bitter gourd	Aerial mycelium brown, colony thick and velvety, concentric zonations present, on the reverse side the colony is black.	Aerial mycelium brown, colony thick and velvety, concentric zonations more conspicuous, on the reverse side the colony is black.
6	Ivy gourd	Aerial mycelium brownish grey, colony thick and velvety, concentric zonations absent, on the reverse side the colony is black.	Aerial mycelium dark brown, colony thick and velvety, concentric zonations absent, on the reverse side the colony is black.
7	Pumpkin	Aerial mycelium brownish grey, colony thick and velvety, concentric zonations present, on the reverse side the colony is black.	-

dark brown aerial mycelium, thick and velvety colony, with or without conspicuous concentric zonations.

4.4.1.2 Sporulation of isolates of Alternaria

The quantitative estimation of spores produced by different isolates of *Alternaria* in pure culture was done by using haemocytometer and the data are presented in Table 2. All the isolates sporulated well in pure culture and the number of conidia produced ranged from 2.5 to 4.9 x 10^{5} conidia ml^{-1}. The highest conidial production (4.9 x 10^{5} spores ml^{-1}) was by the ridge gourd isolate from Vellanikkara. The ivy gourd isolate from Elanad produced the minimum conidia of 2.5 x 10^{5} spores ml^{-1}.

4.4.1.3 Growth rate of different isolates of Alternaria on selected media

Growth rate of different isolates of *Alternaria* was studied on five different media *viz.*, PDA, Host leaf extract agar medium, Richard's agar medium, Czapek (Dox) agar medium and Selective medium for *Alternaria.* The colony diameter of the different isolates of *Alternaria* was recorded at an interval of two days after incubation till the full growth in Petri dishes.

4.4.1.3.1 Ash gourd

Though the time taken for full growth in Petri dishes vary, all the five media supported the growth of *Alternaria* isolated from ash gourd (Table3). On PDA, Vellanikkara isolate took 10 days for the complete growth whereas Elanad isolate took only eight days. However isolates from both locations took 10, 14, 12 and 12 days on Host leaf extract agar medium, Richard's agar medium, Czapek (Dox) agar medium and Selective medium respectively for the full growth.

4.4.1.3.2 Snake gourd

From the data (Table 4) it is evident that all the five different media tested, supported the growth of the two isolates of *Alternaria* from snake gourd. However the

Table 2. Sporulation of different isolates of *Alternaria*

Sl. No.	Isolates	Locations	Number of spores ($x10^5$ ml^{-1}) *
1	Ash gourd	VKA	4.1
		ELA	3.2
2	Snake gourd	VKA	4.5
		ELA	3.7
3	Ridge gourd	VKA	4.9
		ELA	4.0
4	Bottle gourd	VKA	3.6
		ELA	4.5
5	Bitter gourd	VKA	2.7
		ELA	3.3
6	Ivy gourd	VKA	2.8
		ELA	2.5
7	Pumpkin	VKA	3.5

VKA - Vellanikkara

ELA - Elanad

* Average of five observations

Table 3. Effect of selected media on the growth rate of *Alternaria* isolate from ash gourd

Sl. No.	Medium	Locations	Colony diameter (cm)						
			Days after inoculation						
			2	4	6	8	10	12	14
1	PDA	VKA	2.00	5.50	6.50	8.25	9.00		
		ELA	3.00	5.80	8.10	9.00			
2	HLEA	VKA	2.50	4.10	6.43	8.30	9.00		
		ELA	2.90	5.60	7.53	8.06	9.00		
3	RAM	VKA	2.00	3.37	4.50	5.80	6.90	8.80	9.00
		ELA	1.20	2.62	3.20	5.50	7.05	7.90	9.00
4	CAM	VKA	1.70	4.50	6.33	8.15	8.95	9.00	
		ELA	1.70	3.45	4.63	6.14	7.95	9.00	
5	SM	VKA	1.45	2.60	5.60	7.69	8.94	9.00	
		ELA	1.45	3.35	5.10	7.22	8.60	9.00	

PDA - Potato Dextrose Agar Medium

HLEA - Host Leaf Extract Agar Medium

RAM - Richard's Agar Medium

CAM - Czapek (Dox) Agar Medium

SM - Selective Medium

VKA - Vellanikkara

ELA - Elanad

Table 4. Effect of selected media on the growth rate of *Alternaria* isolate from snake gourd

Sl. No.	Medium	Locations	Colony diameter (cm)						
			Days after inoculation						
			2	4	6	8	10	12	14
1	PDA	VKA	2.50	4.70	6.30	8.15	9.00		
		ELA	2.30	4.90	6.70	8.19	9.00		
2	HLEA	VKA	2.05	4.38	7.05	8.38	9.00		
		ELA	2.05	4.73	6.70	8.25	9.00		
3	RAM	VKA	1.45	3.10	4.60	6.05	7.30	9.00	
		ELA	1.45	2.55	3.90	5.00	6.65	8.50	9.00
4	CAM	VKA	1.70	3.33	5.00	5.90	6.90	8.80	9.00
		ELA	1.10	2.43	4.10	5.10	5.95	7.80	9.00
5	SM	VKA	1.45	2.30	4.03	6.30	8.15	9.00	
		ELA	1.45	2.90	4.50	6.65	8.55	9.00	

PDA - Potato Dextrose Agar Medium

HLEA - Host Leaf Extract Agar Medium

RAM - Richard's Agar Medium

CAM - Czapek (Dox) Agar Medium

SM - Selective Medium

VKA - Vellanikkara

ELA -Elanad

time taken to attain full growth varied with the media. On PDA and Host leaf extract agar medium, isolates from both locations took 10 days and 14 and 12 days on Czapek (Dox) agar medium and Selective medium respectively to complete full growth on Petri dishes. Whereas on Richard's agar medium, isolate from Vellanikkara took 12 days and that from Elanad took 14 days to complete full growth.

4.4.1.3.3 Ridge gourd

Alternaria isolate from ridge gourd grew well on all the five different media. Isolate from both the locations took 10, 10, 12 and 12 days on PDA, Host leaf extract agar medium, Czapek (Dox) agar medium and Selective medium respectively. Variation among isolates of different location was noticed only in Richard's agar medium, where isolate from Vellanikkara took 14 days and that from Elanad took 10 days to complete the growth. Difference in growth rate of both the isolates was noticed on all media (Table5).

4.4.1.3.4 Bottle gourd

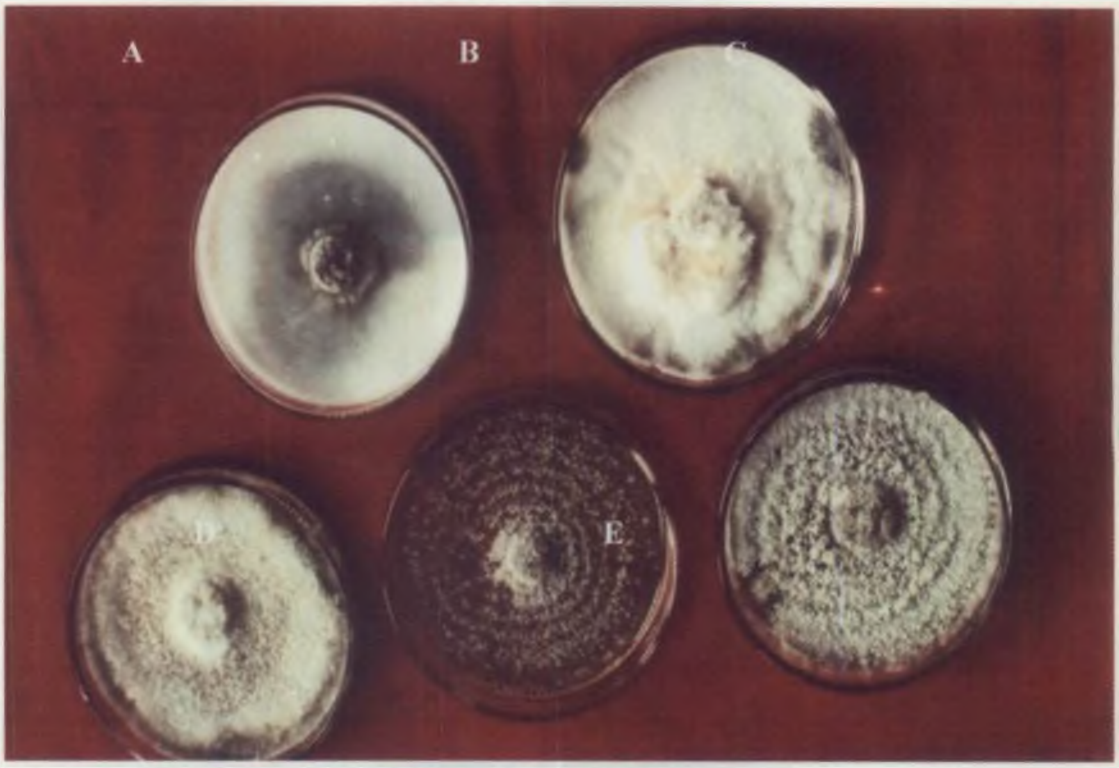
Isolates from both the locations gave good growth on all the five different media (Table6). But on all media variation in growth rate was observed. On PDA Host leaf extract agar medium and Selective medium, isolates from both the locations took 10, 10 and 12 days respectively to complete the full growth in Petri dishes. With regard to growth rate, variability was noticed among isolates of two different locations on Richard's agar medium and Czapek (Dox) agar medium. Isolate from Vellanikkara took 16 and 14 days on Richard's agar medium and Czapek (Dox) agar medium respectively and where those from Elanad took only 14 and 12 days respectively on same media (Plate 5).

4.4.1.3.5 Bitter gourd

The isolate from both the locations produced good growth on all media tested (Table 7). Variation in growth rate among isolates of two different locations was noticed

Plate 5. CULTURAL CHARACTERS

Alternaria **colony of bottle gourd isolate on various media**



A - Potato dextrose agar medium B - Host leaf extract agar medium

C - Czapek (Dox) agar medium D - Richard's agar medium

E - Selective medium for *Alternaria*

Table 5. Effect of selected media on the growth rate of *Alternaria* isolate from ridge gourd

Sl. No.	Medium	Locations	Colony diameter (cm)						
			Days after inoculation						
			2	4	6	8	10	12	14
1	PDA	VKA	2.60	4.90	7.10	8.40	9.00		
		ELA	2.50	4.70	7.50	8.55	9.00		
2	HLEA	VKA	2.40	4.73	7.00	8.30	9.00		
		ELA	2.10	4.40	6.68	8.44	9.00		
3	RAM	VKA	1.50	2.57	3.50	4.90	6.15	7.30	9.00
		ELA	2.00	4.07	6.20	8.20	9.00		
4	CAM	VKA	1.30	3.48	4.33	6.47	8.15	9.00	
		ELA	2.00	3.83	5.47	7.35	8.65	9.00	
5	SM	VKA	1.45	3.53	6.27	7.90	8.94	9.00	
		ELA	1.45	2.60	5.20	7.45	8.94	9.00	

PDA - Potato Dextrose Agar Medium

HLEA - Host Leaf Extract Agar Medium

RAM - Richard's Agar Medium

CAM - Czapek (Dox) Agar Medium

SM - Selective Medium

VKA - Vellanikkara

ELA -Elanad

Table 6. Effect of selected media on the growth rate of *Alternaria* isolate from bottle gourd

Sl. No.	Medium	Locations	Colony diameter (cm)							
			Days after inoculation							
			2	4	6	8	10	12	14	16
1	PDA	VKA	2.30	5.18	7.70	8.65	9.00			
		ELA	2.30	5.05	7.50	8.45	9.00			
2	HLEA	VKA	2.60	4.57	7.00	8.30	9.00			
		ELA	2.00	4.32	6.47	8.24	9.00			
3	RAM	VKA	1.20	2.20	3.07	3.53	5.10	6.60	8.50	9.00
		ELA	2.00	3.47	4.50	5.85	6.85	8.50	9.00	
4	CAM	VKA	2.00	2.88	4.03	5.75	7.35	8.80	9.00	
		ELA	2.00	3.25	4.90	6.90	8.20	9.00		
5	SM	VKA	1.45	2.30	4.30	6.70	8.75	9.00		
		ELA	1.45	2.60	4.30	6.30	7.90	9.00		

PDA - Potato Dextrose Agar Medium

HLEA - Host Leaf Extract Agar Medium

RAM - Richard's Agar Medium

CAM - Czapek (Dox) Agar Medium

SM - Selective Medium

VKA - Vellanikkara

ELA -Elanad

Table 7. Effect of selected media on the growth rate of *Alternaria* isolate from bitter gourd

Sl. No.	Medium	Locations	Colony diameter (cm)							
			Days after inoculation							
			2	4	6	8	10	12	14	16
1	PDA	VKA	1.20	3.02	3.60	6.10	8.25	9.00		
		ELA	2.30	3.93	6.23	7.90	9.00			
2	HLEA	VKA	1.20	2.25	3.03	4.61	6.00	7.50	9.00	
		ELA	1.20	2.22	3.13	4.80	6.08	8.30	9.00	
3	RAM	VKA	1.20	2.20	3.30	3.84	4.45	5.90	7.30	9.00
		ELA	1.60	2.93	3.80	4.87	6.10	7.50	9.00	
4	CAM	VKA	1.20	2.22	2.70	3.60	4.20	5.80	7.40	9.00
		ELA	1.50	3.23	5.03	6.50	7.85	9.00		
5	SM	VKA	1.45	2.50	3.50	5.70	7.45	8.30	9.00	
		ELA	1.45	2.70	4.20	6.30	8.00	9.00		

PDA - Potato Dextrose Agar Medium

HLEA - Host Leaf Extract Agar Medium

RAM - Richard's Agar Medium

CAM - Czapek (Dox) Agar Medium

SM - Selective Medium

VKA - Vellanikkara

ELA -Elanad

in all media except Host leaf extract agar medium where isolates took 14 days to complete full growth. However variability on growth rate of both isolates was noticed among the other media used. Isolates from Vellanikkara took 12, 14, 16 and 16 days on PDA, Selective medium, Czapek (Dox) agar medium and Richard's agar medium respectively for full growth and isolates from Elanad took 10, 12, 12 and 14 days respectively on the same media.

4.4.1.3.6 Ivy gourd

From Table 8, it was found that all five media supported the growth of isolate from both locations. No difference was noticed among isolates of two locations grown on all the five different media, however variation in growth rate was noticed among the different media used and they took 10, 10, 14, 12 and 12 days on PDA, Host leaf extract agar medium, Richard's agar medium, Czapek (Dox) agar medium and Selective medium respectively.

4.4.1.3.7 Pumpkin

Variation was noticed in growth rate among the different media used. However there was no difference in growth rate for the isolate of Vellanikkara on PDA and Host leaf extract agar medium and in both medium full growth was completed in 10 days. Whereas in Selective medium, Czapek (Dox) agar medium and Richard's agar medium it took 12, 14 and 16 days respectively for full growth (Table9).

With regard to the overall performance on growth rate of *Alternaria* on different media (Table 10) it was found that PDA was the best medium for the growth of all isolates. On PDA various isolates took 8-12 days to complete full growth in Petri dishes. It was followed by Host leaf extract agar medium and Selective medium on which they took 10-14 and 12-14 days respectively to complete full growth. Richard's agar medium was found to be the least effective medium for growth of all isolates, on which they took 12-16 days except ridge gourd isolate from Elanad area, which took only 10

Table 8. Effect of selected media on the growth rate of *Alternaria* isolate from ivy gourd

Sl. No.	Medium	Locations	Colony diameter (cm)						
			Days after inoculation						
			2	4	6	8	10	12	14
1	PDA	VKA	2.20	4.57	7.00	8.45	9.00		
		ELA	2.50	4.60	6.70	8.25	9.00		
2	HLEA	VKA	1.90	4.22	6.50	8.25	9.00		
		ELA	1.90	4.27	6.70	8.40	9.00		
3	RAM	VKA	2.20	3.02	4.33	5.89	7.05	8.30	9.00
		ELA	1.20	2.62	3.63	5.03	6.30	7.30	9.00
4	CAM	VKA	2.00	3.70	5.30	7.00	8.55	9.00	
		ELA	2.00	3.40	4.90	6.70	8.25	9.00	
5	SM	VKA	1.45	2.30	4.30	6.70	8.75	9.00	
		ELA	1.45	2.90	4.60	6.80	8.50	9.00	

PDA - Potato Dextrose Agar Medium

HLEA - Host Leaf Extract Agar Medium

SM - Selective Medium

RAM - Richard's Agar Medium

CAM - Czapek (Dox) Agar Medium

VKA - Vellanikkara

ELA - Elanad

Table 9. Effect of selected media on the growth rate of *Alternaria* isolate from pumpkin

Sl. No.	Medium	Location	Colony diameter (cm)							
			Days after inoculation							
			2	4	6	8	10	12	14	16
1	PDA	VKA	2.20	4.80	7.20	8.50	9.00			
2	HLEA	VKA	2.50	4.47	6.77	8.30	9.00			
3	RAM	VKA	1.20	2.10	2.80	4.19	5.83	6.50	8.80	9.00
4	CAM	VKA	1.20	2.70	4.13	5.65	7.30	8.30	9.00	
5	SM	VKA	1.45	2.60	5.60	7.69	8.94	9.00		

PDA - Potato Dextrose Agar Medium

HLEA - Host Leaf Extract Agar Medium

RAM - Richard's Agar Medium

CAM - Czapek(Dox) Agar Medium

SM - Selective Medium

VKA - Vellanikkara

ELA - Elanad

Table 10. Growth in days taken by *Alternaria* isolates on different media

Sl. No.	Isolates	Locations	Days taken to cover full growth in Petri dish				
			PDA	HLEA	RAM	CAM	SM
1	Ash gourd	VKA	10	10	14	12	12
		ELA	8	10	14	12	12
2	Snake gourd	VKA	10	10	12	14	12
		ELA	10	10	14	14	12
3	Ridge gourd	VKA	10	10	14	12	12
		ELA	10	10	10	12	12
4	Bottle gourd	VKA	10	10	16	14	12
		ELA	10	10	14	12	12
5	Bitter gourd	VKA	12	14	16	16	14
		ELA	10	14	14	12	12
6	Ivy gourd	VKA	10	10	14	12	12
		ELA	10	10	14	12	12
7	Pumpkin	VKA	10	10	16	14	12

PDA - Potato Dextrose Agar Medium

HLEA - Host Leaf Extract Agar Medium

RAM - Richard's Agar Medium

CAM - Czapek (Dox) Agar Medium

SM - Selective Medium

VKA - Vellanikkara

ELA -Elanad

days to cover the full growth. Variation in growth rate of the same isolate from the two locations was observed on Richard's agar medium, on which all isolates except ash gourd and ivy gourd took different time to complete the full growth in Petri dishes. The isolates from bottle gourd and bitter gourd of Vellanikkara location took the maximum time of 16 days and ridge gourd of Elanad area took minimum time of 10 days to complete the growth on Richard's agar medium.

Among the various media used, variation in growth rate between the same isolates of different location was observed. Variation was observed in case of ash gourd and bitter gourd on PDA, bottle gourd and bitter gourd on Czapek (Dox) agar medium, and bitter gourd on Selective medium, whereas on Host leaf extract agar medium no variation on growth rate of the same isolates of different locations was observed.

Among the various isolates of *Alternaria*, ash gourd isolate from Elanad area took the minimum time of 8 days on PDA to complete the growth. The isolates from snake gourd, ridge gourd, bottle gourd, ivy gourd and pumpkin took 10 days on PDA and Host leaf extract agar medium to cover the full growth in Petri dishes. Whereas the bitter gourd isolate showed variability in growth rate on different media, which took minimum of 10-12 days on PDA to maximum of 14-16 days on Richard's agar medium to cover the full growth.

4.4.2 Morphological characters of isolates of *Alternaria*

Morphological characters of different isolates of *Alternaria* such as conidiophore production, conidial characters and their microscopic measurements were studied in detail. The variations in morphological characters were also studied with Euclidean co- efficient and were clustered by the Unweighed Pair Group Average Method (UPGMA) using NTSYS pc 2.02 software to produce grouping. The results are presented in Table 11 to 16.

4.4.2.1 *Production of conidiophores in culture*

All isolates of *Alternaria* were found to produce conidiophore in pure culture. Conidiophores arise singly or in group, branched, straight, cylindrical, and tapering towards the apex, septate, brown, and smooth with conidial scar at the tip (Plate 6A).

4.4.2.2 *Size of conidial chain of Alternaria isolates*

The isolates of *Alternaria* from two locations produced conidia in chain (Table11). The size of conidial chain ranged from 2 to 12. *Alternaria* isolates from ash gourd and snake gourd from Elanad and the isolate from ridge gourd and bottle gourd from Vellanikkara produced maximum conidia of 12 in chain (Plate 6B).

4.4.2.3 *Morphological characters and microscopic measurements*

The descriptions of morphological characters and microscopic measurements of various structures of different isolates of *Alternaria* are given in the Table 12 to 14.

4.4.2.4 *Cluster analysis of Alternaria isolates based on morphological characters*

Genetic dissimilarity index (DI) of *Alternaria* isolates from the two locations was computed from morphological characters as Euclidean co- efficient using NTSYS pc 2.02 software. The results are presented in Table 15. The dendrogram was constructed by using Unweighed Pair Group Average Method (UPGMA) as shown in Fig15 .The lowest dissimilarity of 4.54 was observed between isolates of ash gourd (VKA) and snake gourd (VKA). It was followed by 5.63 between isolates of bitter gourd (VKA) and ivy gourd (VKA). Highest dissimilarity of 35.97 was observed between isolates of ash gourd (VKA) and bitter gourd (ELA) followed by 35.56 between isolates of snake gourd (ELA) and bottle gourd (ELA). The isolates were grouped in to four clusters A, B, C, D based on the morphological characters (Table 16). Cluster A was further divided in to three sub clusters A_1, A_2 and A_3. Clusters B, C and D had one sub cluster each. In sub cluster A_2, the isolates ash gourd (VKA) and snake gourd (VKA) recorded the lowest dissimilarity index (4.54). In sub cluster A_1, the isolates of bitter gourd (VKA) and ivy gourd (VKA) had the lowest dissimilarity index (5.63) and it was followed by the isolates ash gourd

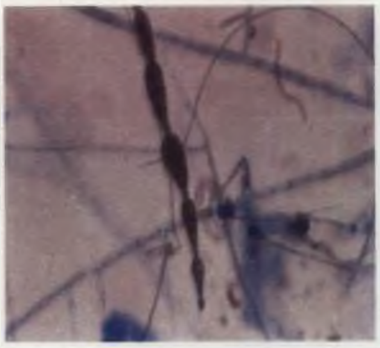
Plate 6. MORPHOLOGICAL CHARACTERS

A. Conidiophores

(400 X)

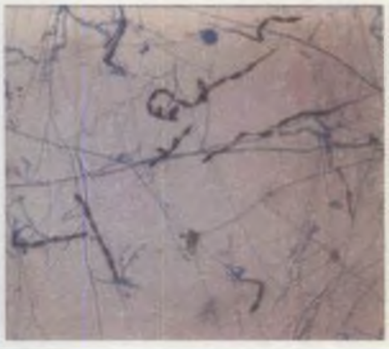
(400 X)

B. Conidial chain



(400 X)

(400 X)



(100 X)

Table 11. Size of conidial chain of *Alternaria* isolates

Sl. No.	Isolates	Locations	Size of Conidial chain
1	Ash gourd	VKA	2-4
		ELA	2-12
2	Snake gourd	VKA	2-4
		ELA	2-12
3	Ridge gourd	VKA	2-12
		ELA	2-4
4	Bottle gourd	VKA	2-12
		ELA	2-4
5	Bitter gourd	VKA	2-4
		ELA	2-6
6	Ivy gourd	VKA	2-6
		ELA	2-4
7	Pumpkin	VKA	2-4
		ELA	2-4

VKA - Vellanikkara

ELA - Elanad

Table 12. Morphological descriptions of different isolates of *Alternaria* from different cucurbitaceous vegetables

Sl. No.	Isolates	Locations	
		Vellanikkara	Elanad
1	Ash gourd	Hyphae branched, brownish grey with 4.06µm breadth, septate at 16.12-28.21µm intervals, conidiophores branched, seen in single or groups and having 80.6-112.84 µm length with 3-9 septa, conidia formed in chain, straight, obclavate, smooth and verruculose, brown, 16.24-48.72 x 4.06-16.24µm and with 3-7 transverse and 1-2 longitudinal septa, beak prominent measuring 4.06-24.36 x 4.06µm with 1-2 septa (Plate 7a, Fig.1).	Hyphae branched, brownish grey with 4.06µm breadth, septate at 20.3-28.42µm intervals, conidiophores branched, seen in single or groups and having 81.2-101.5 µm length with 3-7 septa, conidia formed in chain, straight, obclavate, smooth and verruculose, brown, 28.42-60.9 x 4.06-12.18µm and with 3-8 transverse and 1-4 longitudinal septa, beak prominent measuring 4.06-20.3 x 4.06µm with 1-3 septa (Plate 8a, Fig.8).
2	Snake gourd	Hyphae branched, brownish grey with 4.06µm breadth, septate at 16.12-28.21µm intervals, conidiophores branched, seen in single or groups and having 80.6-108.51 µm length with 3-7 septa, conidia formed in chain, straight, obclavate, smooth and verruculose, brown, 16.24-52.78 x 4.06-12.18µm and with 2-5 transverse and 1-2 longitudinal septa, beak prominent measuring 4.06-36.54 x 4.06µm with 1-2 septa (Plate 7b, Fig.2).	Hyphae branched, brown with 4.06µm breadth, septate at 20.3-32.48µm intervals, conidiophores branched, seen in single or groups and having 101.5-113.68 µm length with 3-9 septa, conidia formed in branched chain, straight, obclavate, smooth, brown, 28.42-60.9 x 4.06-12.18µm and with 3-5 transverse and 1-2 longitudinal septa, beak prominent measuring 4.06-24.36 x 4.06µm with 1-2 septa (Plate 8b, Fig.9).

Sl. No.	Isolates	Locations	
		Vellanikkara	Elanad
3	Ridge gourd	Hyphae branched, brownish grey with 4.03µm breadth, septate at 16.12-28.21µm intervals, conidiophores branched, seen in single or groups and having 80.6-104.78 µm length with 3-9 septa, conidia formed in chain, straight , obclavate, smooth and verruculose, brown, 12.09-76.57 x 4.03-12.09µm and with 3-6 transverse and 1-4 longitudinal septa, beak prominent measuring 4.03-20.15 x 4.03-8.06µm with 1-2 septa (Plate 7c, Fig. 3).	Hyphae branched, brownish grey with 4.06µm breadth, septate at 20.3-28.42µm intervals, conidiophores branched, seen in single or groups and having 52.78-113.68 µm length with 3-6 septa, conidia formed in chain, straight, obclavate, smooth and verruculose, brown, 20.3-44.66 x 4.06-12.18µm and with 3-6 transverse and 1-2 longitudinal septa, beak prominent measuring 4.06-12.18 x 4.06µm with 1-2 septa (Plate 8c, Fig.10).
4	Bottle gourd	Hyphae branched, brownish grey with 4.03µm breadth, septate at 16.12-28.21µm intervals, conidiophores branched, seen in single or groups and having 80.6-100.75 µm length with 3-6 septa, conidia formed in chain, straight, obclavate, smooth and verruculose, brown, 12.09-52.39 x 4.03-16.12µm and with 3-6 transverse and 1-5 longitudinal septa, beak prominent measuring 4.03-20.15 x 4.03µm with 1-2 septa (Plate 7d, Fig.4).	Hyphae branched, brownish grey with 4.06µm breadth, septate at 20.3-28.42µm intervals, conidiophores branched, seen in single or groups and having 48.72-101.5 µm length with 3-7 septa, conidia formed in chain, straight, obclavate, smooth and verruculose, brown, 12.18-73.08 x 4.06-12.18µm and with 2-7 transverse and 1-2 longitudinal septa, beak prominent measuring 4.06-44.66 x 4.06µm with 1-2 septa (Plate 8d, Fig.11).

Sl. No.	Isolates	Locations	
		Vellanikkara	Elanad
5	Bitter gourd	Hyphae branched, brown with 4.03μm breadth, septate at 16.12-32.24μm intervals, conidiophores branched, seen in single or groups and having 80.6-96.72 μm length with 3-6 septa, conidia formed in branched chain, straight, obclavate, smooth, brown, 12.09-76.57 x 4.03-16.12μm and with 3-6 transverse and 1-3 longitudinal septa, transverse septum thick and darker than longitudinal septum, beak prominent measuring 4.03-36.27 x 4.03μm with 1-3 septa (Plate 7e, Fig.5).	Hyphae branched, brown with 4.06μm breadth, septate at 20.3-32.48μm intervals, conidiophores branched, seen in single or groups and having 52.78-101.5 μm length with 3-6 septa, conidia formed in branched chain, straight, obclavate, smooth, brown, 20.3-81.20 x 4.06-12.18μm and with 3-7 transverse and 1-2 longitudinal septa, transverse septum thick and darker than longitudinal septum, beak prominent measuring 4.06-60.90 x 4.06μm with 1-3 septa (Plate 8e, Fig.12).
6	Ivy gourd	Hyphae branched, brownish grey with 4.03μm width, septate at 16.12-28.21μm intervals, conidio-phores branched, seen in single or groups and having 80.6-104.78 μm length with 3-7 septa, conidia formed in branched chain, straight, obclavate, smooth, brown, 20.15-80.60 x 4.03-12.09μm and with 3-6 transverse and 1-4 longitudinal septa, beak prominent measuring 4.03-20.15 x 4.03μm with 1-2 septa (Plate 7f, Fig.6).	Hyphae branched, brownish grey with 4.06μm width, septate at 20.3-28.42μm intervals, conidiophores branched, seen in single or groups and having 48.72-101.5 μm length with 3-7 septa, conidia formed in chain, straight, obclavate, smooth and verruculose, brown, 20.3-89.32 x 4.06-16.24μm and with 2-4 transverse and 1-4 longitudinal septa, beak prominent measuring 4.06-48.72 x 4.06μm with 1-3 septa (Plate 8f, Fig.13).

Sl. No.	Isolates	Locations	
		Vellanikkara	Elanad
7	Pumpkin	Hyphae branched, brownish grey with 4.03μm breadth, septate at 16.12-28.21μm intervals, conidio-phores branched, seen in single or groups and having 80.6-92.69μm length with 3-6 septa, conidia formed in chain, straight, obclavate, smooth and verruculose, brown, 16.12-56.42 x 4.03-12.09μm and with 3-5 transverse and 1-4 longitudinal septa, beak prominent measuring 4.03-20.15 x 4.03-8.06μm with 1-3 septa (Plate 7g, Fig.7).	Hyphae branched, brownish grey with 4.06μm breadth, septate at 20.3-32.48μm intervals, conidio-phores branched, seen in single or groups and having 81.2-113.68μm length with 3-6 septa, conidia formed in chain, straight, obclavate, smooth and verruculose, brown, 24.36-64.96 x 4.06-8.12μm and with 3-6 transverse and 1-2 longitudinal septa, beak prominent measuring 4.06-44.66 x 4.06μm with 1-3 septa (Plate 8g, Fig.14).

Table 13. Morphological characters of different isolates of *Alternaria* from Vellanikkara

Sl. No.	Host/ Isolate	Hypha		Conidiophore			Conidia						Beak		
		Breadth (µm)	Distance between two septa (µm)	Length (µm)	Breadth (µm)	No. of septa	Length (µm)	Breadth (µm)			Number of septum		Length (µm)	Breadth (µm)	No. of septa
								Tip	Middle	Base	Trans verse	Longit udinal			
1	Ash gourd	4.06	16.12-28.21	80.6-112.84	4.06	3-9	16.24-48.72	4.06-16.24	4.06-16.24	4.06-16.24	3-7	1-2	4.06-24.36	4.06	1-2
2	Snake gourd	4.06	16.12-28.21	80.6-108.51	4.06	3-7	16.24-52.78	4.06-12.18	8.12-12.18	4.06-12.18	2-5	1-2	4.06-36.54	4.06	1-2
3	Ridge gourd	4.06	16.12-28.21	80.6-104.78	4.06	3-9	12.09-76.57	4.03-12.09	4.03-12.09	4.03-8.06	3-6	1-4	4.03-20.15	4.03-8.06	1-2
4	Bottle gourd	4.03	16.12-28.21	80.6-100.75	4.03	3-6	12.09-52.39	4.03-16.12	4.03-16.12	4.03-12.09	3-6	1-5	4.03-20.15	4.03	1-2
5	Bitter gourd	4.03	16.12-32.24	80.6-96.72	4.03	3-6	12.09-76.57	4.03-12.09	4.03-16.12	4.03-8.06	3-6	1-3	4.03-36.27	4.03	1-3
6	Ivy gourd	4.03	16.12-28.21	80.6-104.78	4.03	3-7	20.15-80.60	4.03-12.09	4.03-12.09	4.03-12.09	3-6	1-4	4.03-20.15	4.03	1-2
7	Pumpkin	4.03	16.12-28.21	80.6-92.69	4.03	3-6	16.12-56.42	8.06-12.09	4.03-12.09	4.03-20.15	3-5	1-4	4.03-20.15	4.03-8.06	1-3

Mean of 50 observations

Table 14. Morphological characters of different isolates of *Alternaria* from Elanad

Sl. No.	Host/ Isolate	Hypha		Conidiophore			Conidia						Beak		
		Breadth (µm)	Distance between two septa (µm)	Length (µm)	Breadth (µm)	No. of septa	Length (µm)	Breadth (µm)			Number of septum		Length (µm)	Breadth (µm)	No. of septa
								Tip	Middle	Base	Transverse	Longitudinal			
1	Ash gourd	4.06	20.30-28.42	81.2-101.5	4.06	3-7	28.42-60.9	8.12-12.18	8.12-12.18	4.06-12.18	3-8	1-4	4.06-20.3	4.06	1-3
2	Snake gourd	4.06	20.3-32.48	101.5-113.68	4.06	3-9	28.42-60.9	8.12	8.12-12.18	4.06-8.12	3-5	1-2	4.06-24.36	4.06	1-2
3	Ridge gourd	4.06	20.3-28.42	52.78-113.68	4.06	3-6	20.3-44.66	4.06-12.18	4.06-12.18	4.06-8.12	3-6	1-2	4.06-12.18	4.06	1-2
4	Bottle gourd	4.06	20.3-28.42	48.72-101.5	4.06	3-7	12.18-73.08	8.12-12.18	8.12-12.18	4.06-8.12	2-7	1-2	4.06-44.66	4.06	1-2
5	Bitter gourd	4.06	20.3-32.48	52.78-101.5	4.06	3-6	20.3-81.20	4.06-12.18	4.06-12.18	4.06-8.12	3-7	1-2	4.06-60.90	4.06	1-3
6	Ivy gourd	4.06	20.3-28.42	48.72-101.5	4.06	3-7	20.3-89.32	8.12-16.24	8.12-16.24	4.06-12.18	2-4	1-4	4.06-48.72	4.06	1-3
7	Pumpkin	4.06	20.3-32.48	81.2-113.68	4.06	3-6	24.36-64.96	8.12	4.06-8.12	4.06-8.12	3-6	1-2	4.06-44.66	4.06	1-3

Mean of 50 observations

Plate 7. Conidia of various *Alternaria* isolates from Vellanikkara

a. Ash gourd (1000 X)

b. Snake gourd (1000 X)

c. Ridge gourd (1000 X)

d. Bottle gourd (1000 X)

e. Bitter gourd (1000 X)

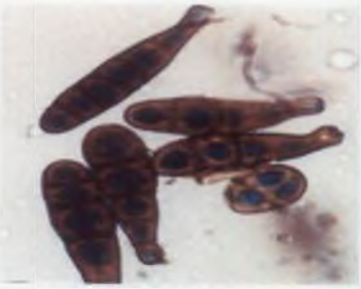
f. Ivy gourd (1000 X)

g. Pumpkin (1000 X)

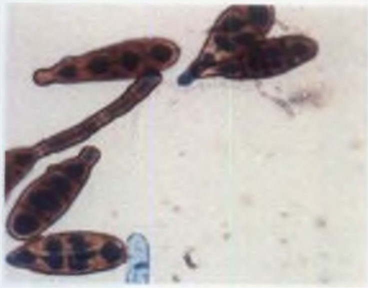
Plate 8. Conidia of various *Alternaria* isolates from Elanad

a. Ash gourd (1000 X)

b. Snake gourd (1000 X)



c. Ridge gourd (1000 X)



d. Bottle gourd (1000 X)

e. Bitter gourd (1000 X)

f. Ivy gourd (1000 X)

g. Pumpkin (1000X)

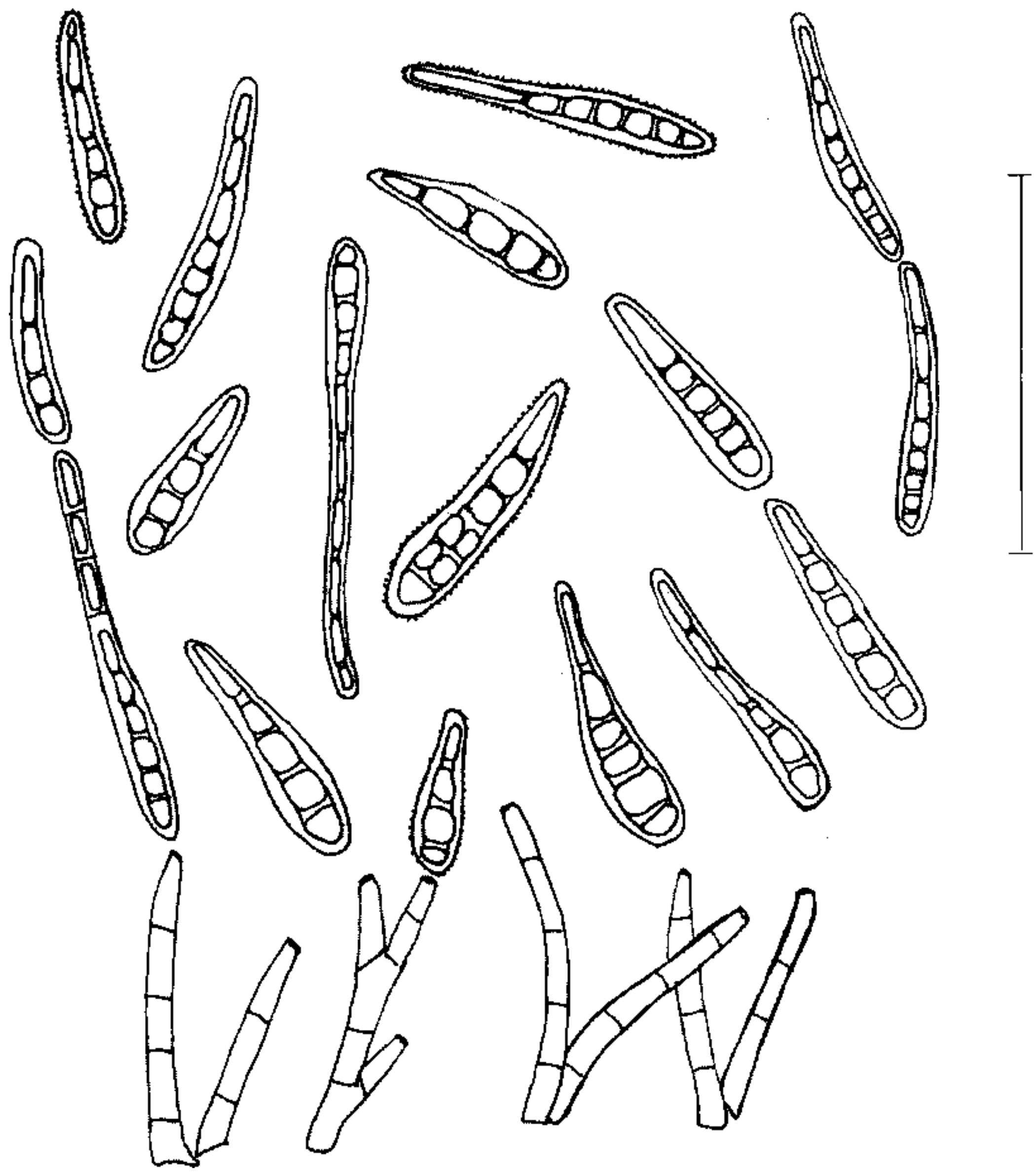


Fig. 1. Conidia and conidiophores of *A. alternata* of ash gourd isolate from Vellanikkara

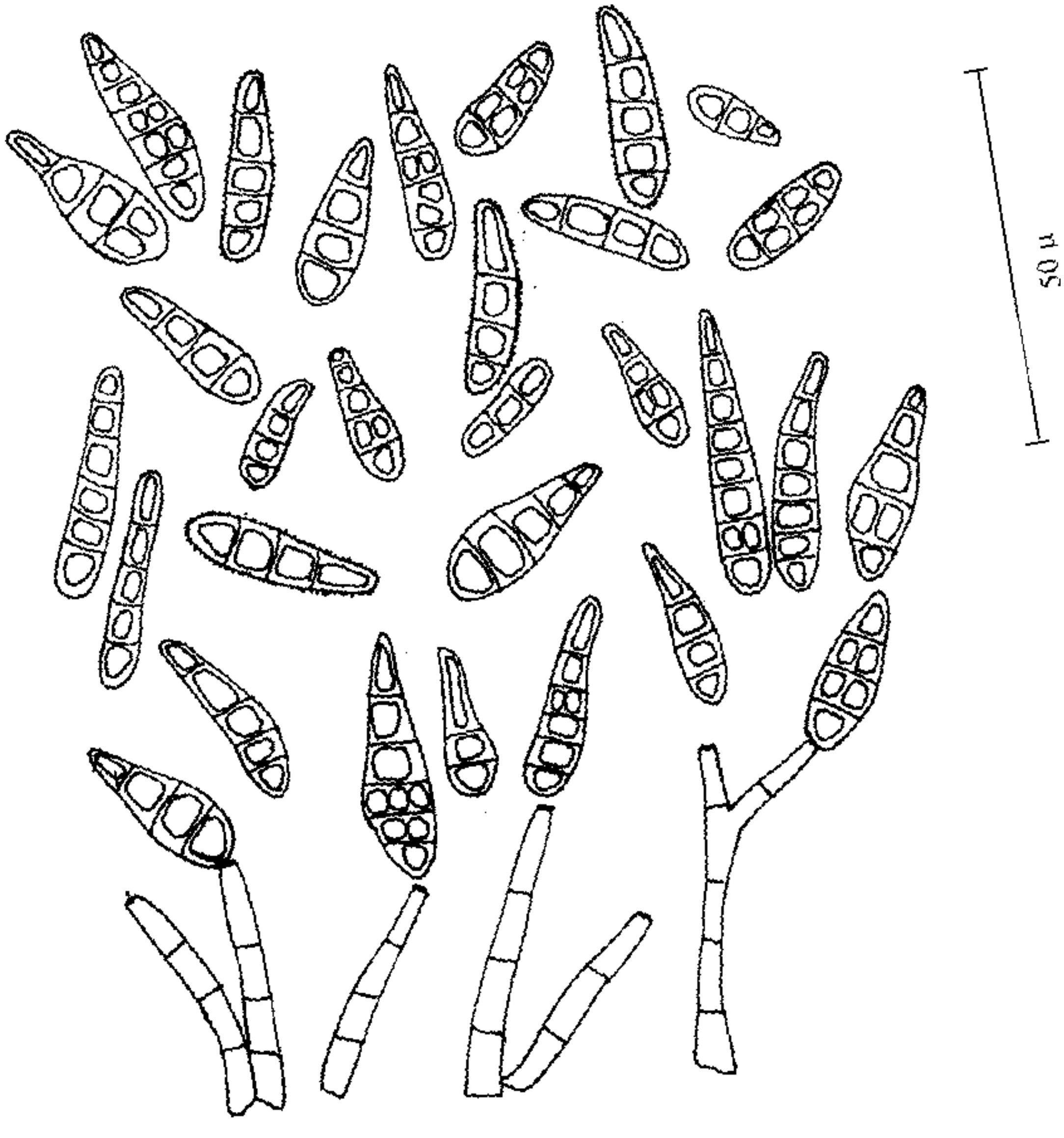


Fig. 2. Conidia and conidiophores of *A. alternata* of snake gourd isolate from Vellanikkara

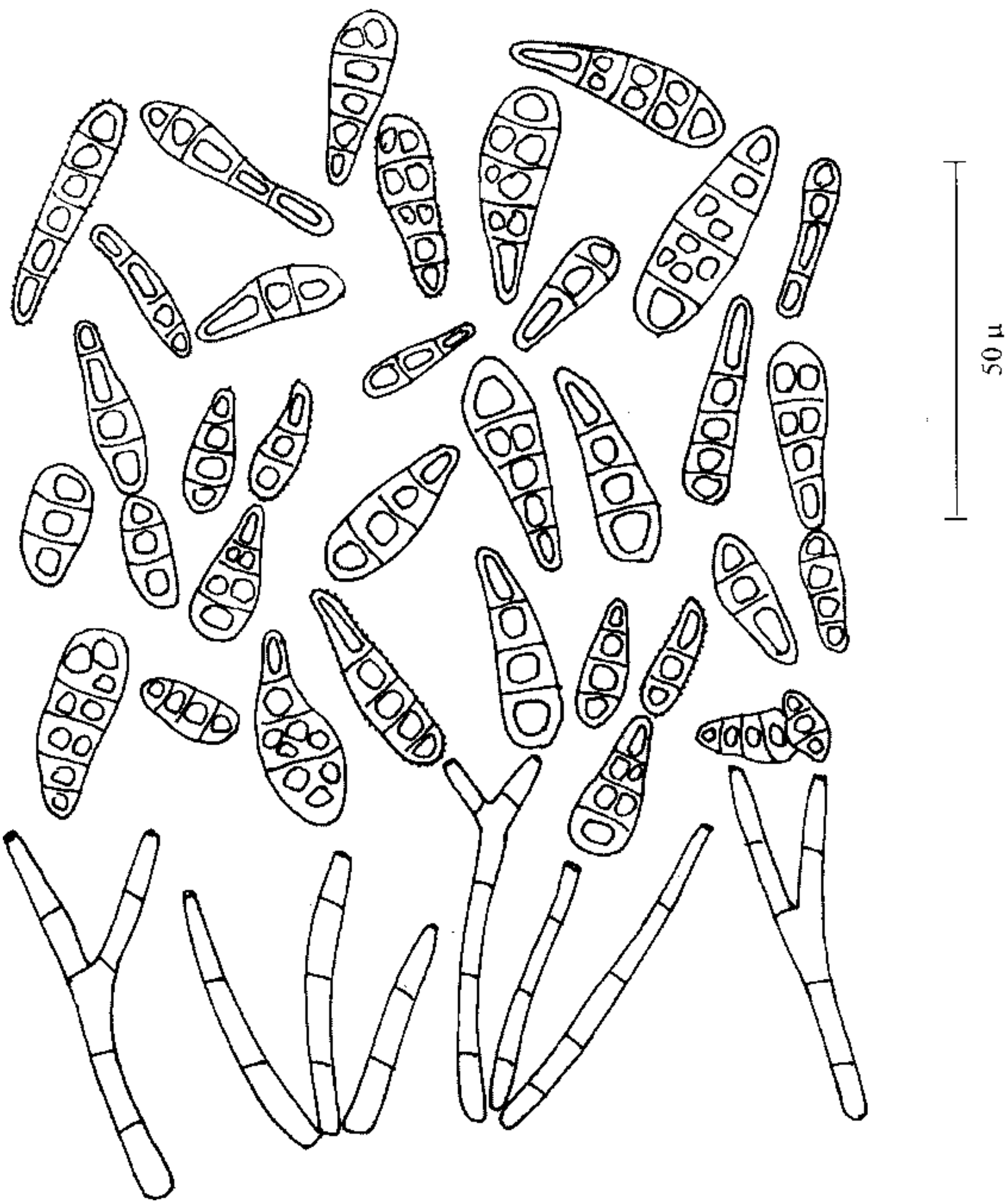


Fig. 3. Conidia and conidiophores of *A. alternata* of ridge gourd isolate from Vellanikkara

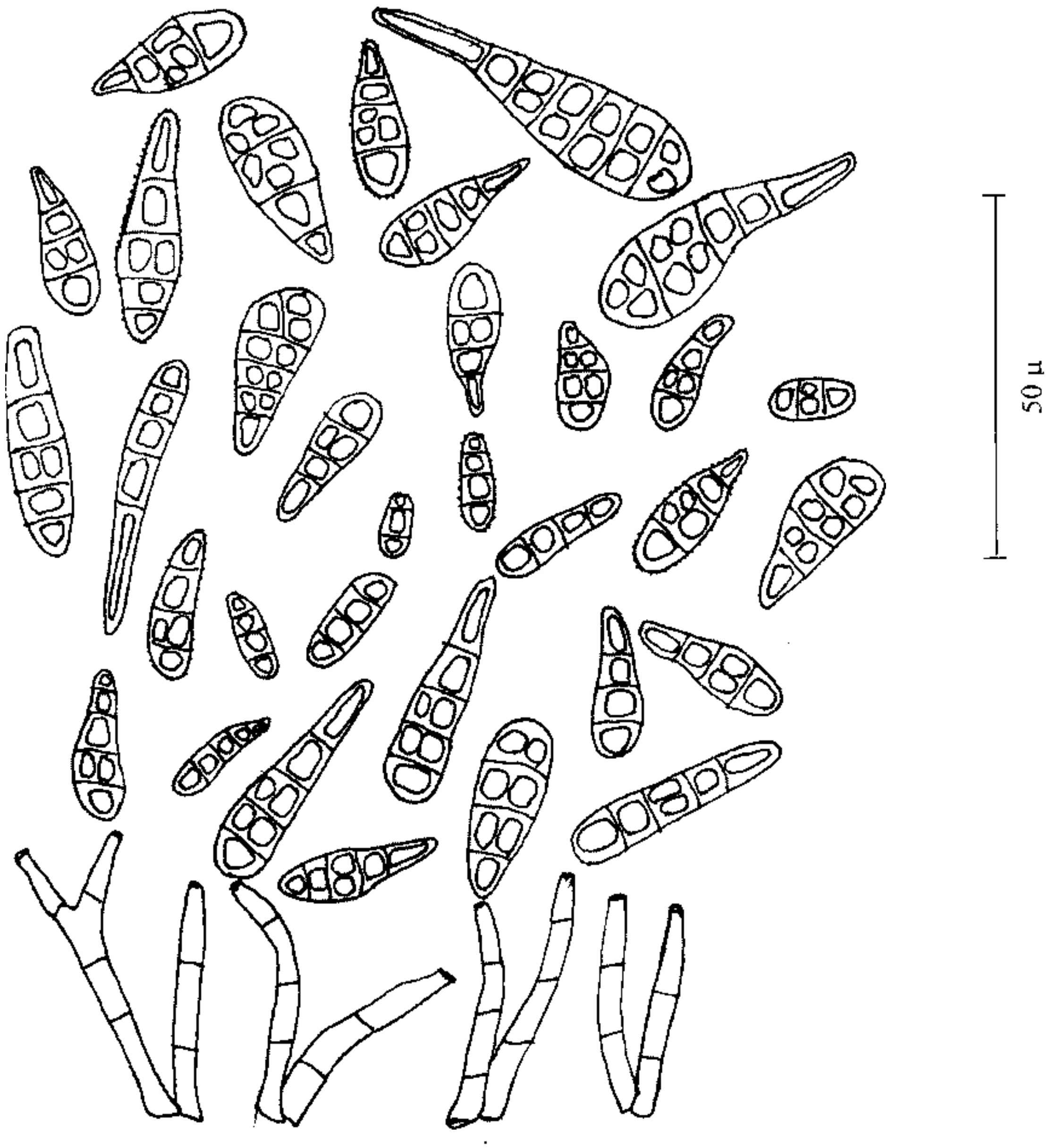


Fig. 4. Conidia and conidiophores of *A. alternata* of bottle gourd isolate from Vellanikkara

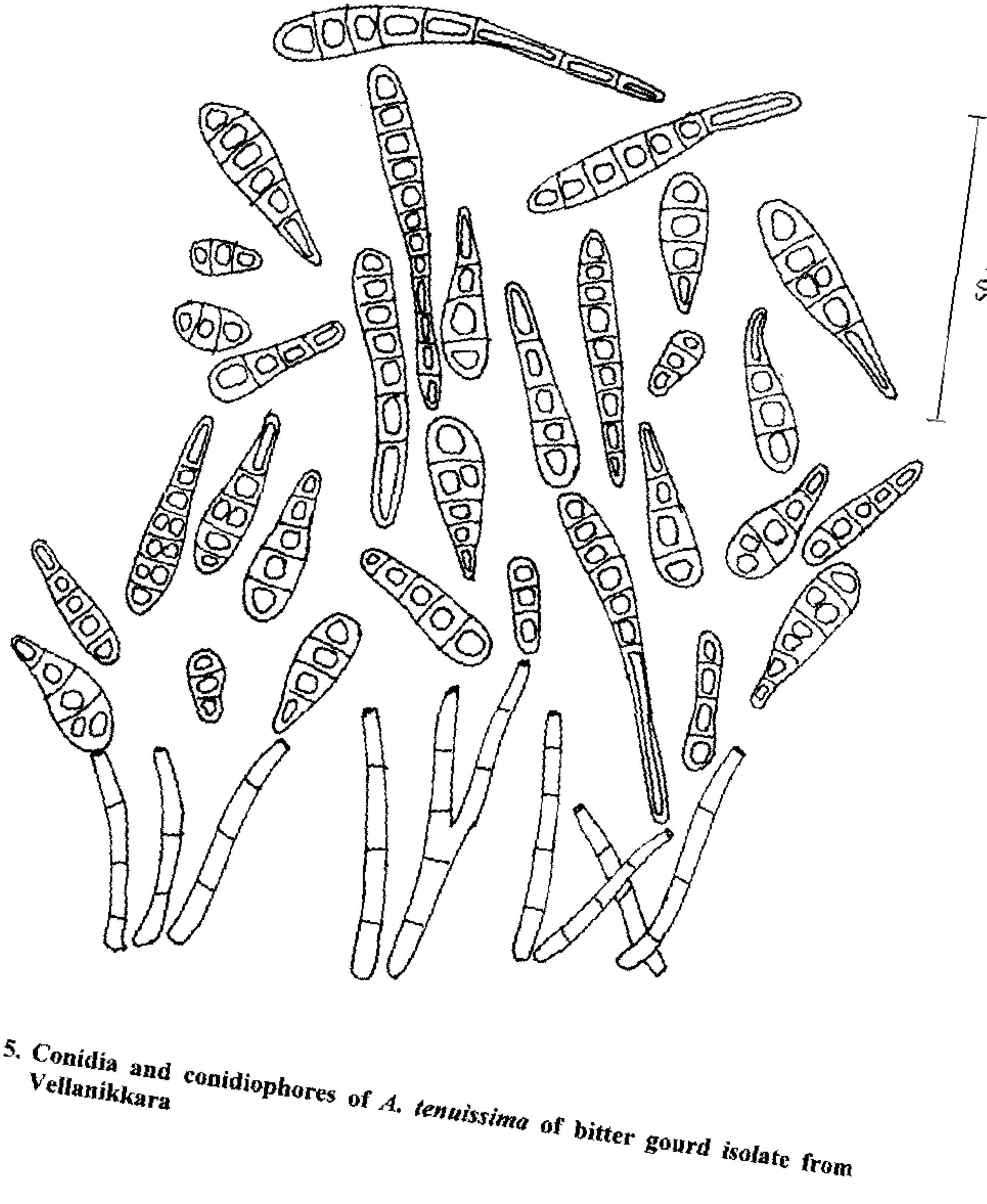


Fig. 5. Conidia and conidiophores of *A. tenuissima* of bitter gourd isolate from Vellanikkara

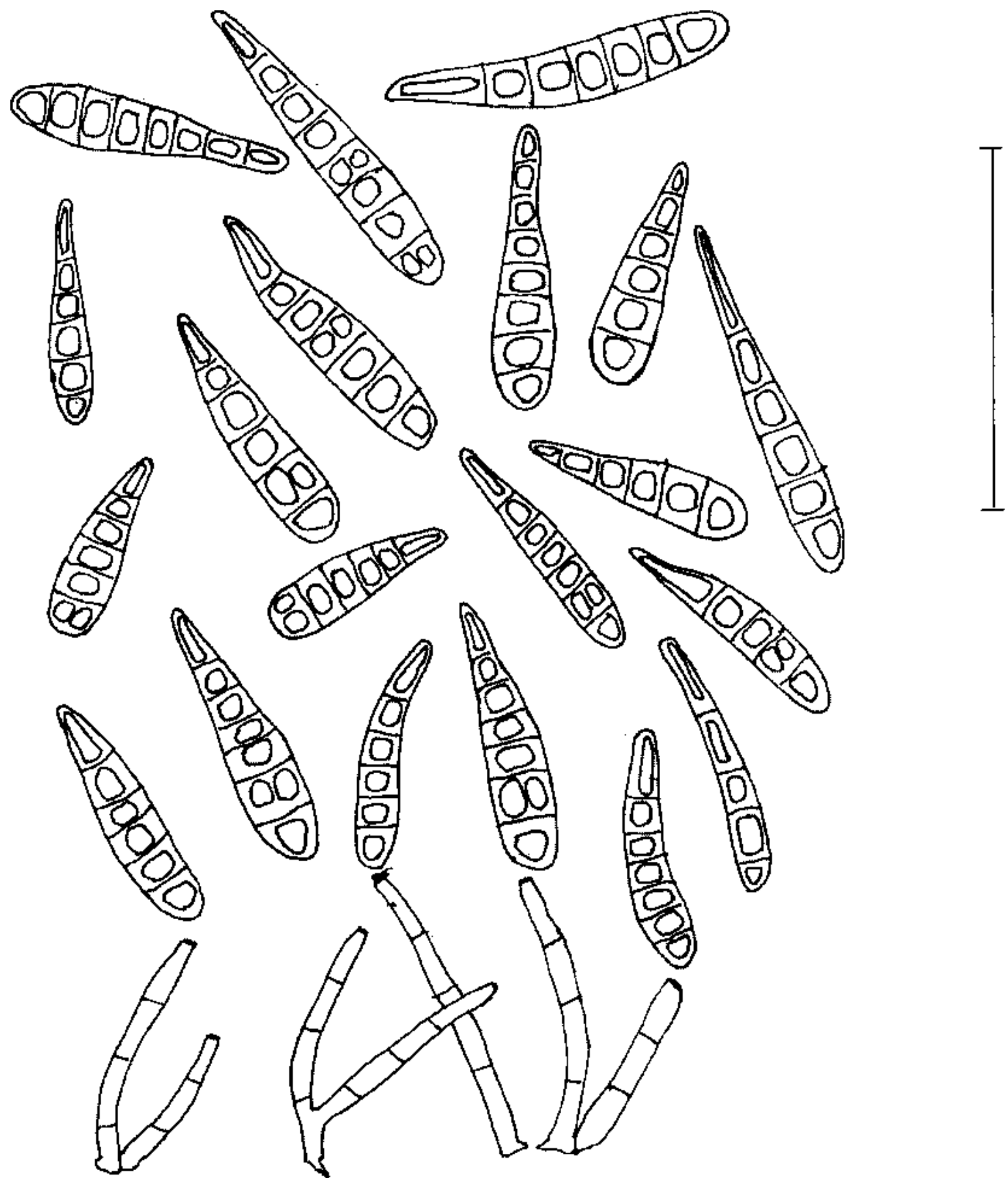


Fig. 6. Conidia and conidiophores of *A. tenuissima* of ivy gourd isolate from Vellanikkara

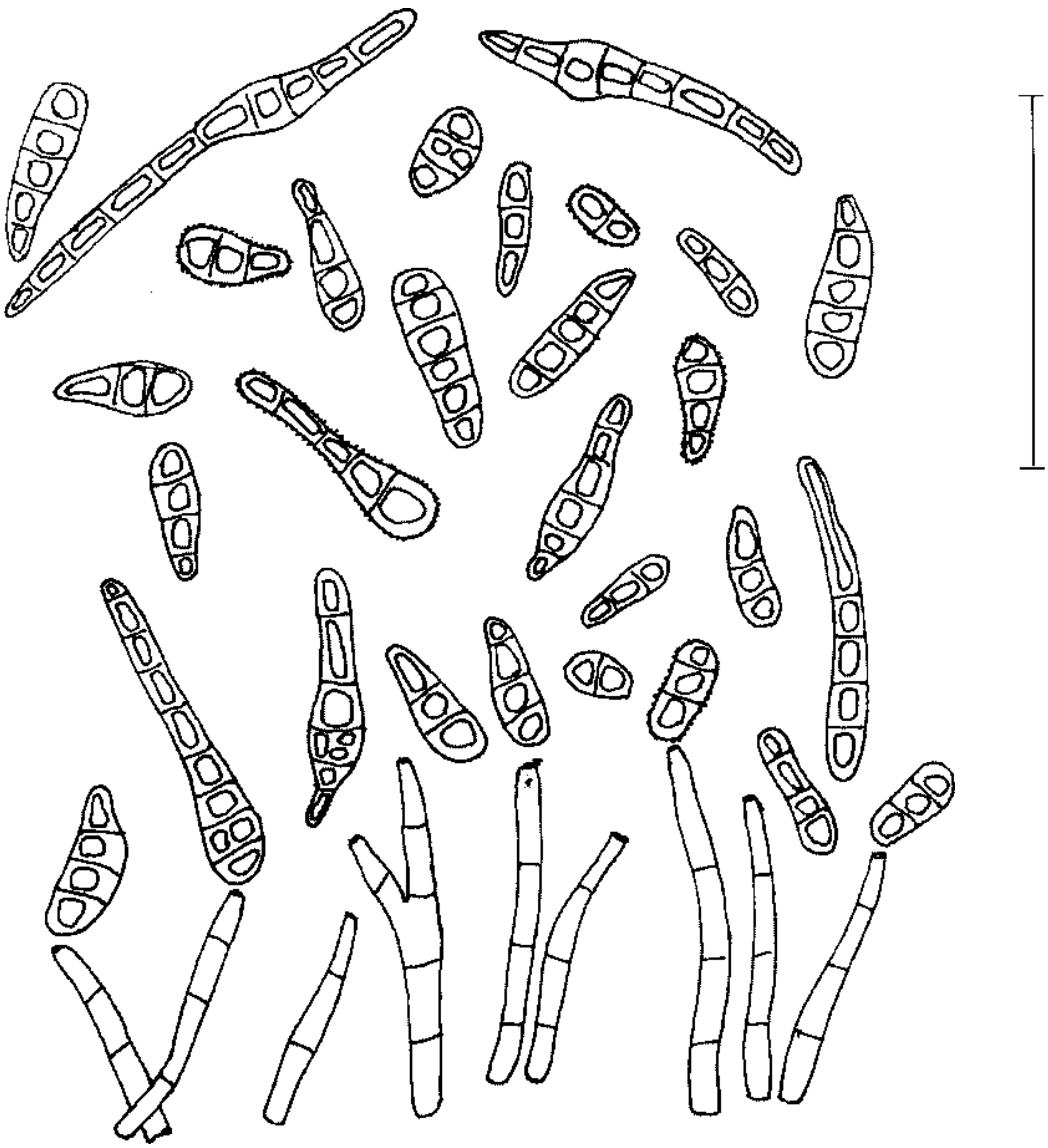


Fig. 7. Conidia and conidiophores of *A. alternata* of pumpkin isolate from Vellanikkara

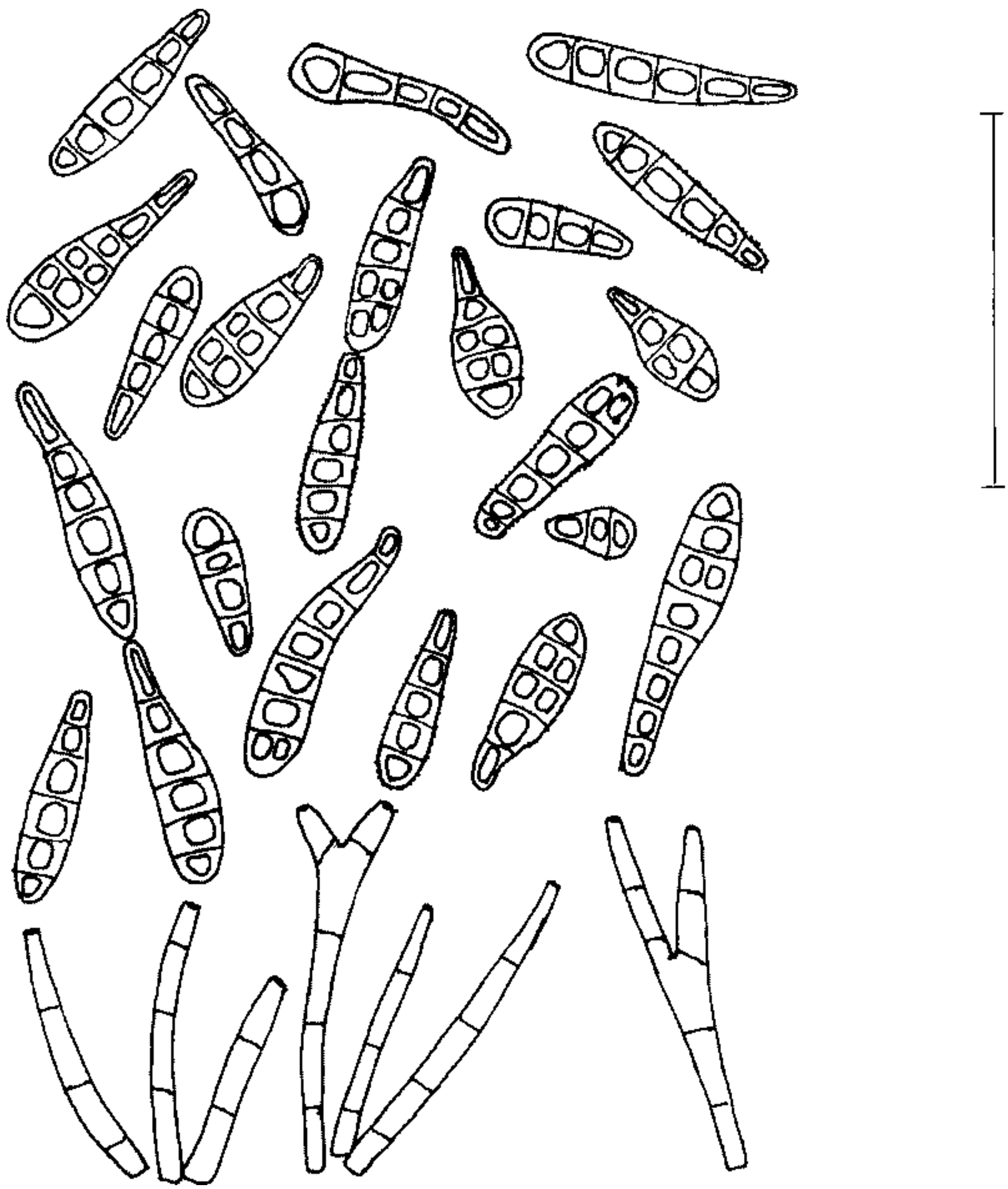


Fig. 8. Conidia and conidiophores of *A. alternata* of ash gourd isolate from Elanad

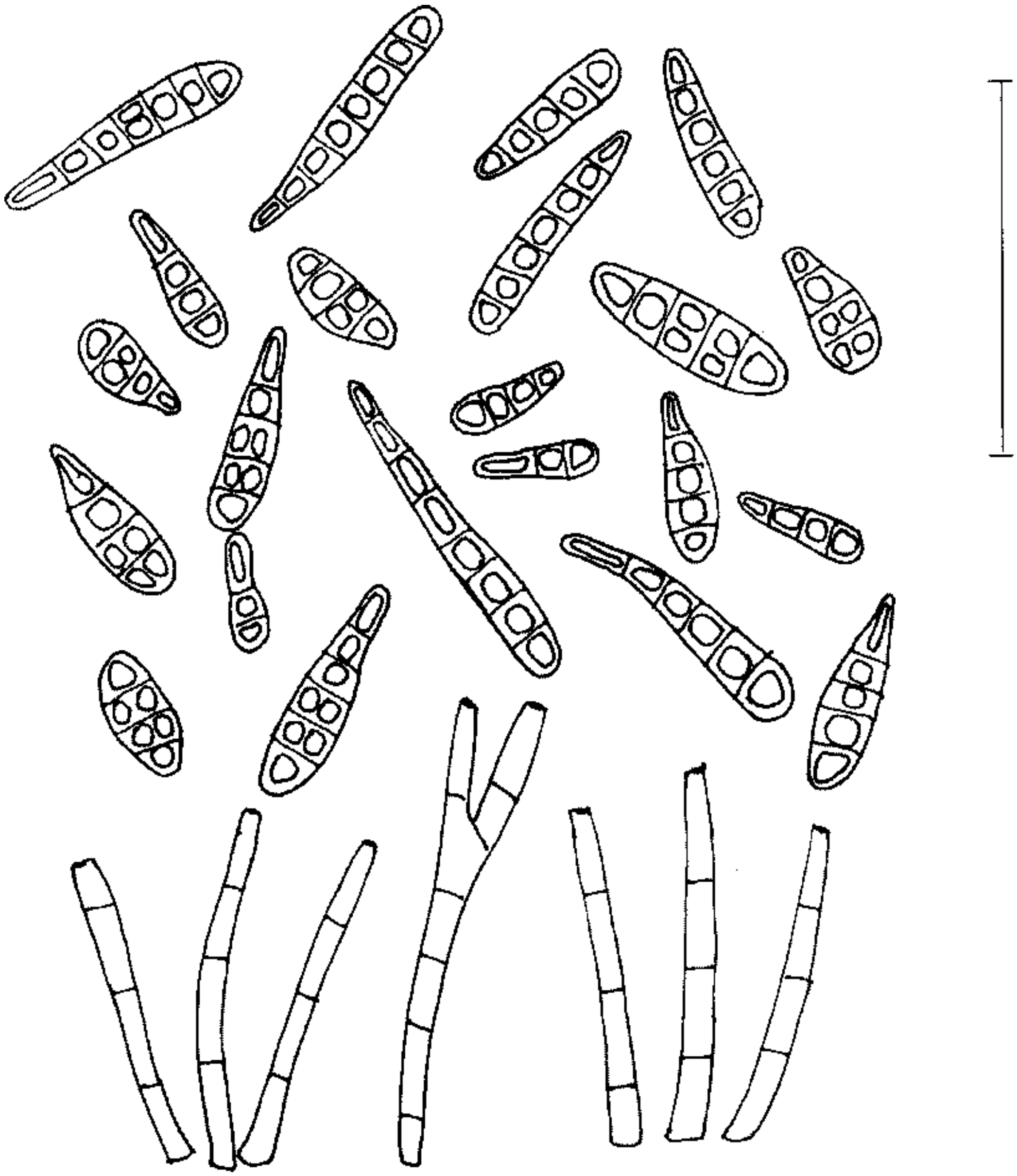


Fig. 9. Conidia and conidiophores of *A. tenuissima* of snake gourd from Elanad

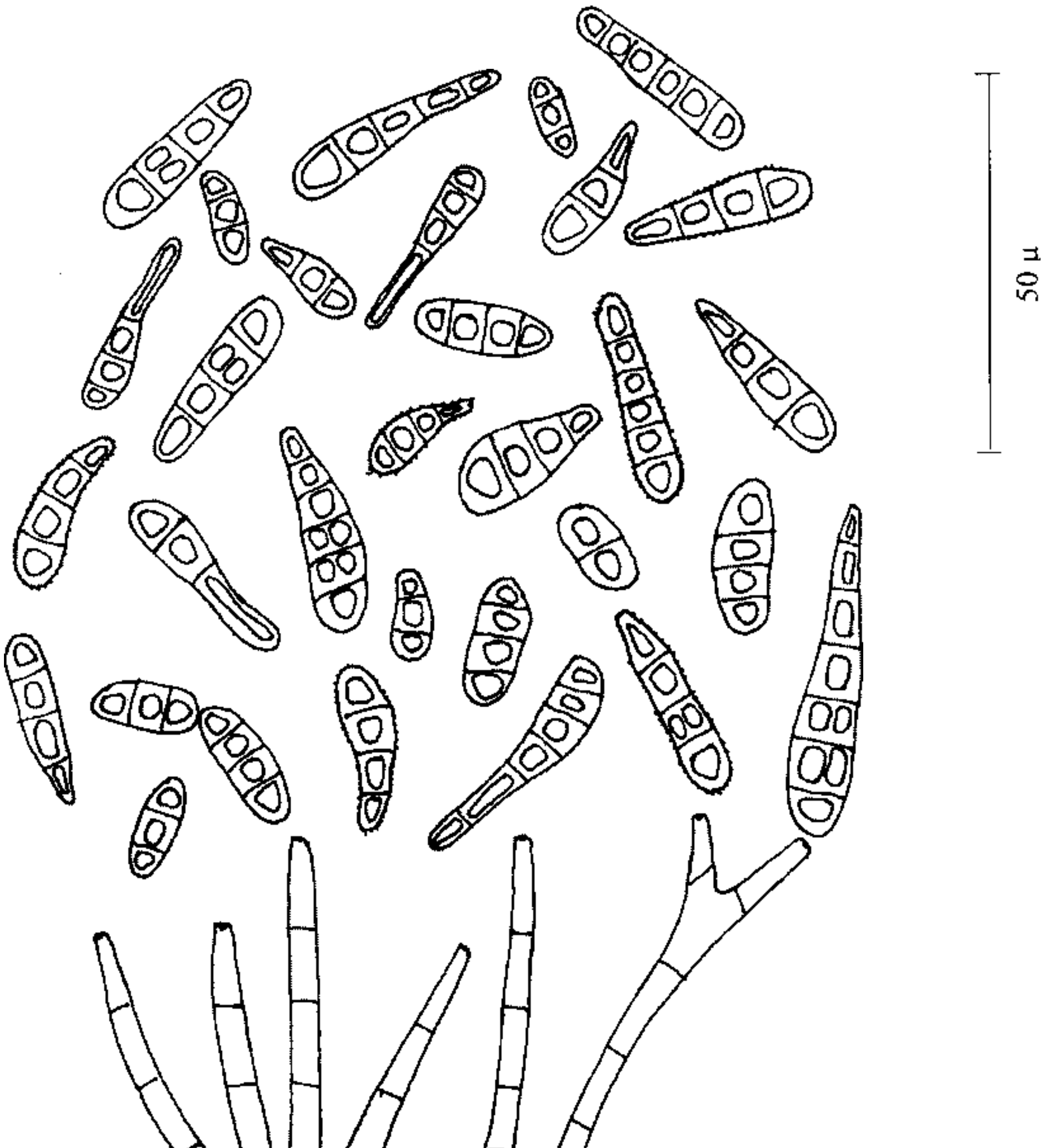


Fig. 10. Conidia and conidiophores of *A. alternata* of ridge gourd isolate from Elanad

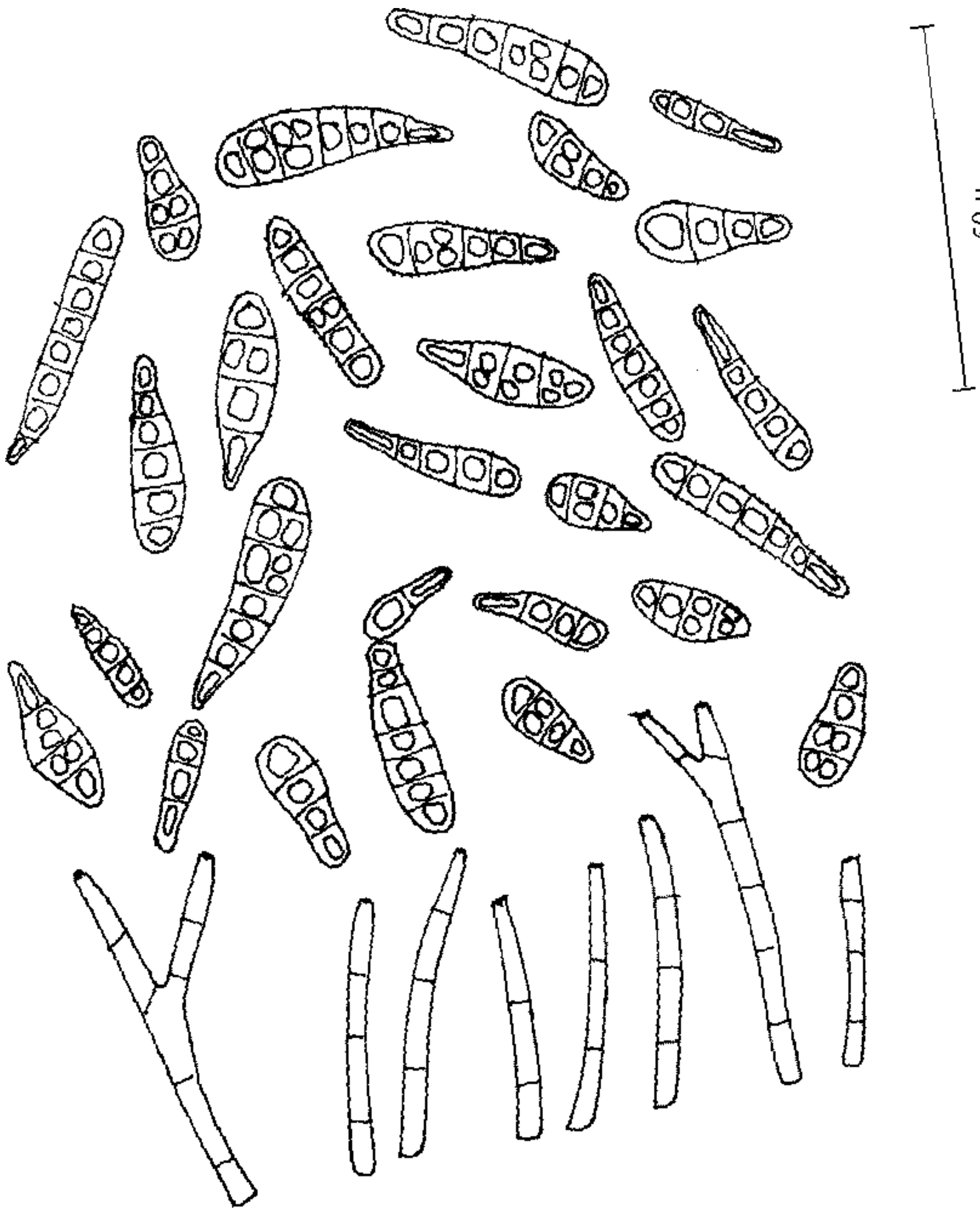


Fig. 11. Conidia and conidiophores of *A. alternata* of bottle gourd isolate from Elanad

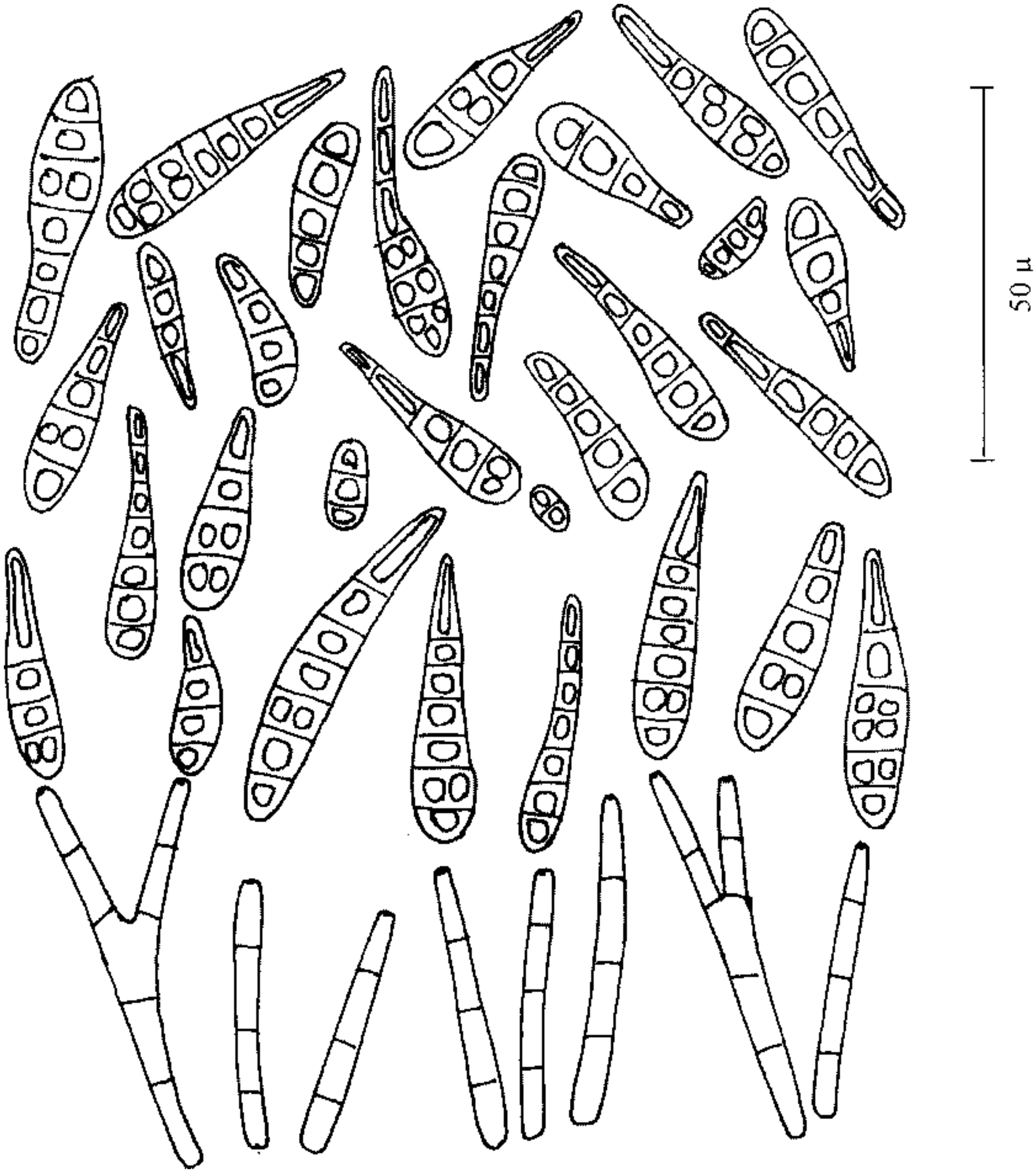


Fig. 12. Conidia and conidiophores of *A. tenuissima* of bitter gourd isolate from Elanad

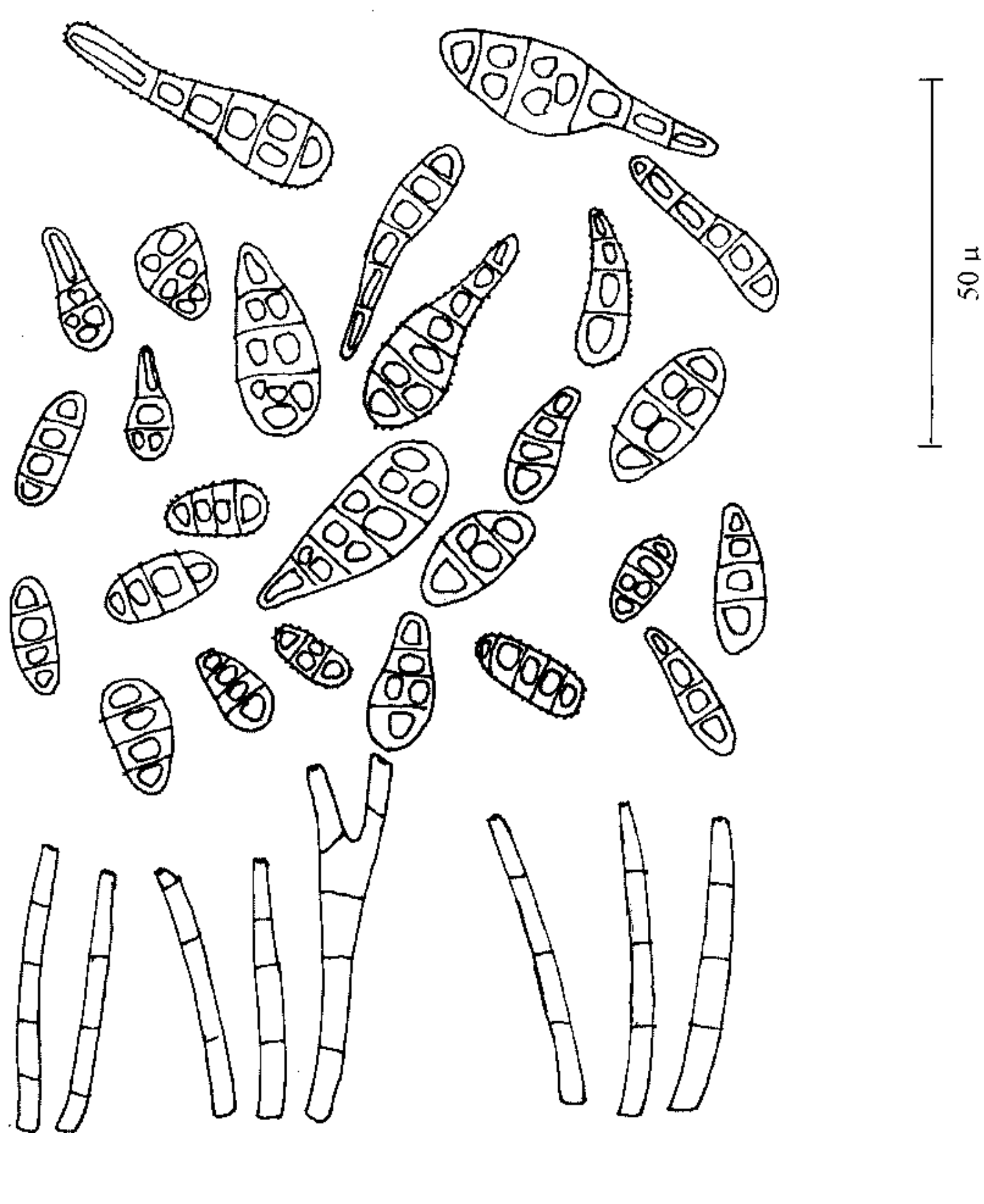


Fig. 13. Conidia and conidiophores of *A. alternata* of ivy gourd isolate from Elanad

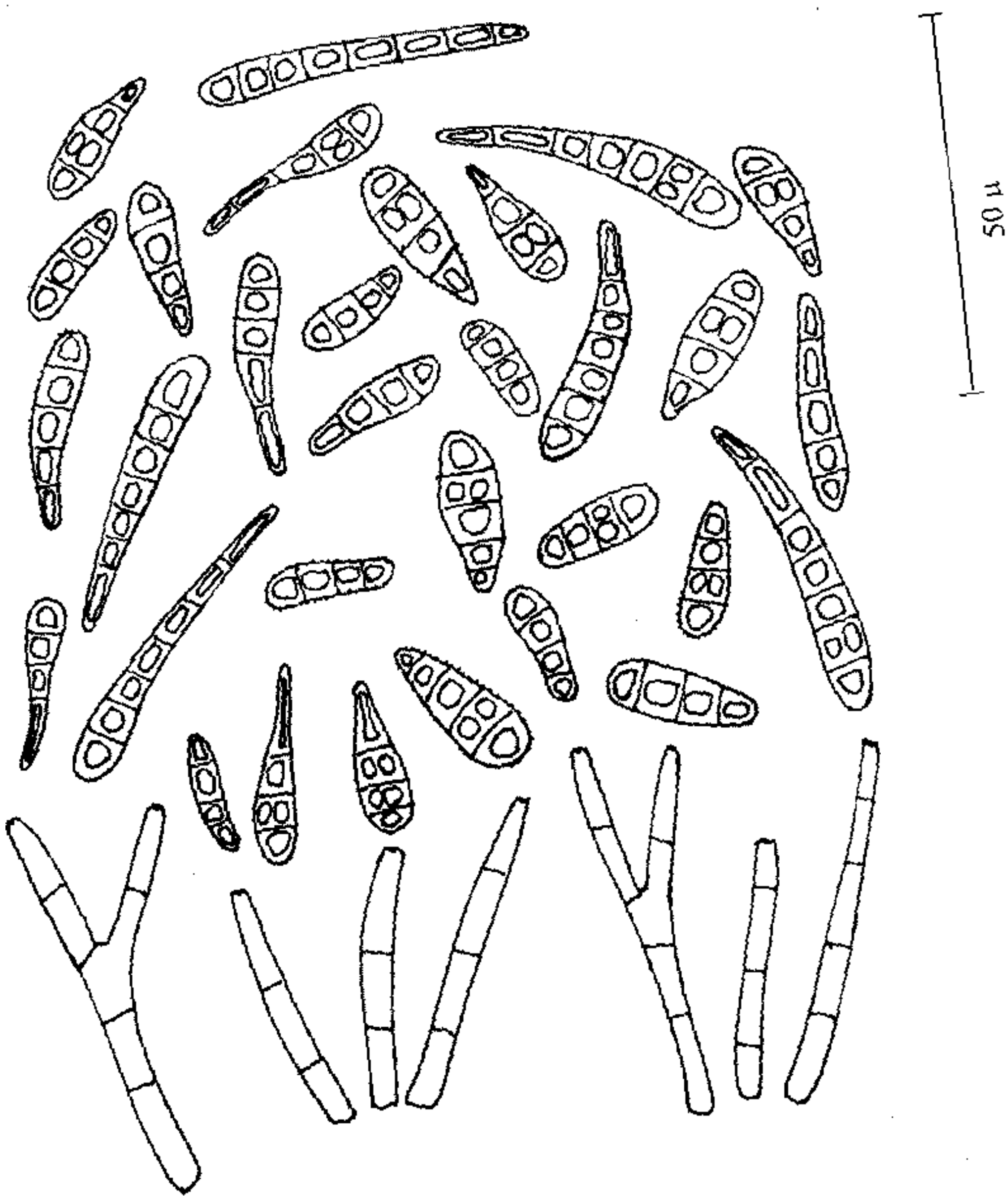


Fig. 14. Conidia and conidiophores of *A. alternata* of pumpkin isolate from Elanad

Table 15. Dissimilarity matrix of *Alternaria* isolates from the two locations based on morphological characters

	Ash (ELA)	Ash (VKA)	Snake (ELA)	Snake (VKA)	Ridge (ELA)	Ridge (VKA)	Bottle (ELA)	Bottle (VKA)	Bitter (ELA)	Bitter (VKA)	Ivy (ELA)	Ivy (VKA)	Pump (ELA)	Pump (VKA)
Ash (ELA)	0													
Ash (VKA)	14.71	0												
Snake(ELA)	18.08	19.87	0											
Snake (VKA)	12.72	4.54	20.14	0										
Ridge (ELA)	13.43	14.71	28.30	12.43	0									
Ridge (VKA)	5.95	12.80	17.06	10.57	14.32	0								
Bottle (ELA)	20.94	22.46	35.56	21.09	10.09	21.67	0							
Bottle (VKA)	9.57	9.75	22.28	7.73	10.56	9.35	18.73	0						
Bitter (ELA)	25.28	35.97	33.80	34.19	29.16	25.94	27.51	31.28	0					
Bitter (VKA)	8.52	19.08	20.00	17.04	17.08	8.49	21.82	13.73	19.85	0				
Ivy (ELA)	17.85	25.93	32.71	23.62	14.70	19.14	12.23	19.65	17.50	15.25	0			
IVY (VKA)	6.85	17.26	16.56	14.98	17.74	5.81	24.41	12.40	23.77	5.63	19.11	0		
Pump (ELA)	9.31	8.70	13.71	8.42	15.39	8.68	23.37	10.79	30.68	14.57	23.57	12.03	0	
Pump (VKA)	10.61	12.69	24.70	10.17	7.70	10.58	15.13	7.35	28.38	13.05	16.04	13.21	12.97	0

Table 16. Grouping of *Alternaria* isolates from the two locations based on dissimilarity index for morphological characters

Cluster	Sub cluster	Isolates
A	A_1	Ash gourd (ELA), Ridge gourd (VKA), Bitter gourd (VKA), Ivy gourd (VKA)
	A_2	Ash gourd (VKA), Snake gourd (VKA), Pumpkin (ELA)
	A_3	Ridge gourd (ELA), Bottle gourd (VKA), Pumpkin (VKA)
B	B_1	Bottle gourd (ELA), Ivy gourd (ELA)
C	C_1	Snake gourd (ELA)
D	D_1	Bitter gourd (ELA)

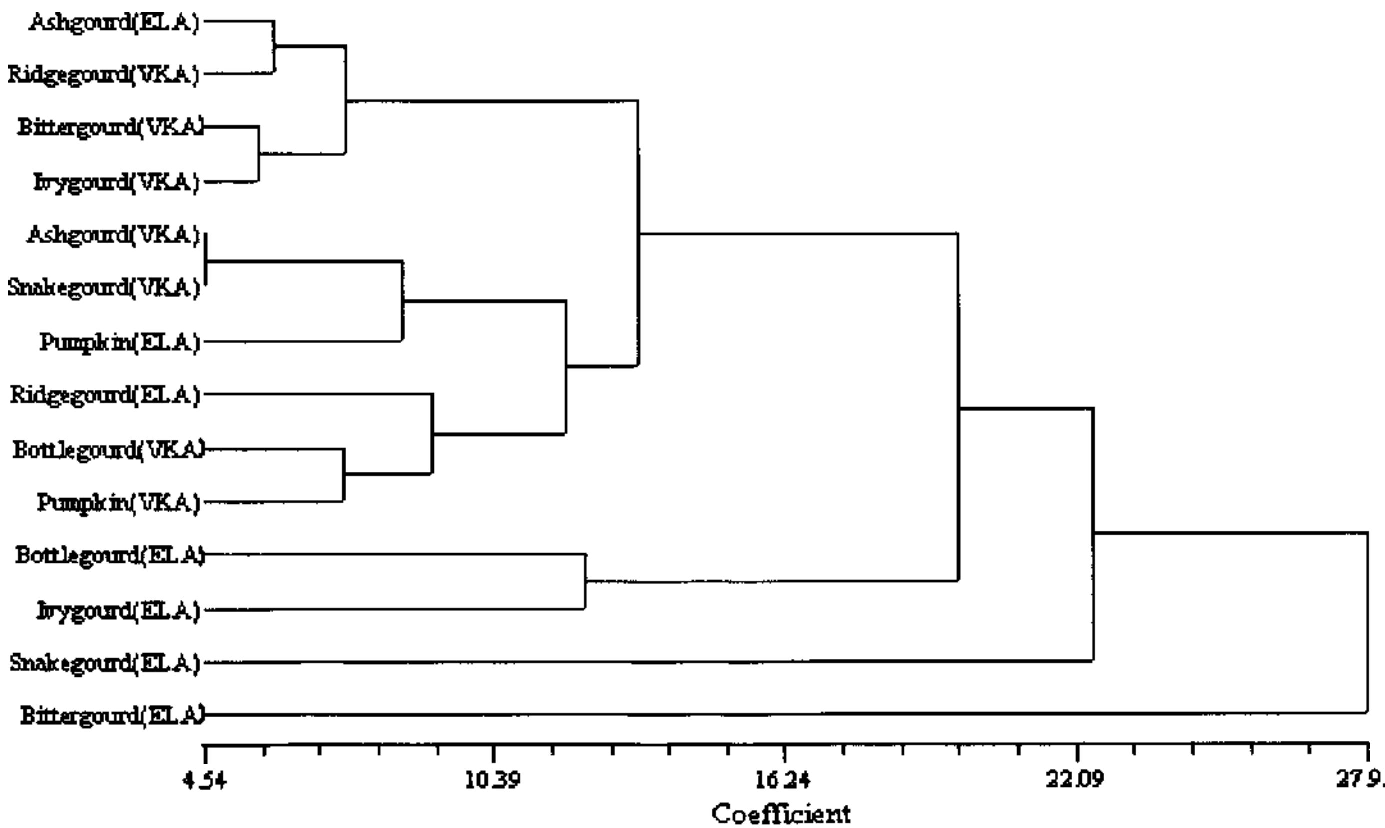


Fig. 15. Unweighed pair group average method (UPGMA) dendrogram based on morphological characters of *Alternaria* isolates from the two locations

(ELA) and ridge gourd (VKA) with a dissimilarity index of 5.95. The isolates of bottle gourd (ELA) and ivy gourd (ELA) were grouped under the sub cluster B_1 of cluster B showed a dissimilarity index of 12.23. The highest dissimilarity index (35.97) was showed by the isolate bitter gourd (ELA) placed under the sub cluster D_1 of cluster D.

4.4.2.5 Identification of different isolates of Alternaria

Based on the cultural and morphological characters different isolates of *Alternaria* from different cucurbitaceous vegetables were identified and the results are presented in Table 17.

Variation was observed among species of *Alternaria* causing leaf blight in different cucurbitaceous hosts of two different locations in Trichur district. *A. alternata* was the only species infecting ash gourd, ridge gourd, bottle gourd and pumpkin, while in bitter gourd *A. tenuissima* was identified as the causal organism of leaf blight disease. However in snake gourd and ivy gourd both species of *Alternaria* cause leaf blight symptoms. So the two species of *Alternaria viz.*, *A. alternata* and *A. tenuissima* were found to be producing leaf blight in various cucurbitaceous vegetables.

4.5 PHYSIOLOGICAL AND NUTRITIONAL CHARACTERS

The effect of temperature, p^H, light, carbon and nitrogen sources on the growth of different isolates of *Alternaria* was studied.

4.5.1 Effect of temperature on the fresh and dry weight of isolates of *Alternaria*

The effect of three temperatures on the fresh and dry weight of mycelium of isolates of *Alternaria* from different locations was studied.

4.5.1.1 Isolates of Alternaria from Vellanikkara

The data (Table 18) revealed that the over all effect of temperature on different isolates of *Alternaria* was maximum at 25°C (12.01g) and was on par with the

Table 17. Identified *Alternaria* spp. from different locations

Sl.No.	Isolates	Locations	
		Vellanikkara	Elanad
1	Ash gourd	*Alternaria alternata* (Fr.) Keissler.	*Alternaria alternata* (Fr.) Keissler
2	Snake gourd	*Alternaria alternata* (Fr.) Keissler	*Alternaria tenuissima* (Kunze ex Pers.) Wiltshire
3	Ridge gourd	*Alternaria alternata* (Fr.) Keissler	*Alternaria alternata* (Fr.) Keissler
4	Bottle gourd	*Alternaria alternata* (Fr.) Keissler	*Alternaria alternata* (Fr.) Keissler
5	Bitter gourd	*Alternaria tenuissima* (Kunze ex Pers.) Wiltshire	*Alternaria tenuissima* (Kunze ex Pers.) Wiltshire
6	Ivy gourd	*Alternaria tenuissima* (Kunze ex Pers.) Wiltshire	*Alternaria alternata* (Fr.) Keissler
7	Pumpkin	*Alternaria alternata* (Fr.) Keissler	*Alternaria alternata* (Fr.) Keissler

Table 18. Effect of temperature on fresh and dry weight of isolates of *Alternaria* from Vellanikkara.

Sl. No.	Isolates	*Fresh weight (g)				*Dry weight (g)			
		25°C	30°C	35°C	Mean	25°C	30°C	35°C	Mean
1	Ash gourd	7.81^{efgh}	9.88^{cdefg}	$11.93B^{cdef}$	9.87^{c}	0.62^{def}	0.55^{ef}	0.90^{abcd}	0.69^{b}
2	Snake gourd	14.70^{ab}	12.30^{bcd}	10.83^{bcdef}	12.61^{ab}	1.12^{a}	0.95^{abc}	0.68^{cdef}	0.91^{a}
3	Ridge gourd	12.39^{bcd}	10.86^{bcdef}	9.41^{cdefgh}	10.89^{bc}	0.85^{abcde}	0.80^{bcdef}	0.54^{f}	0.73^{b}
4	Bottle gourd	16.83^{a}	13.67^{abc}	8.97^{defgh}	13.16^{a}	0.96^{abc}	0.66^{cdef}	0.82^{abcdef}	0.81^{ab}
5	Bitter gourd	12.16^{bcde}	11.79^{bcdef}	5.77^{gh}	9.91^{c}	0.88^{abcd}	0.87^{abcd}	0.70^{cdef}	0.82^{ab}
6	Ivy gourd	7.64^{fgh}	8.80^{defgh}	5.55^{h}	7.33^{d}	0.76^{bcdef}	0.77^{bcdef}	0.67^{cdef}	0.73^{b}
7	Pumpkin	12.51^{bcd}	9.93^{cdefg}	6.19^{gh}	9.54^{c}	1.01^{ab}	0.74^{bcdef}	0.68^{cdef}	0.81^{ab}
	Mean	12.01^{a}	11.03^{a}	8.38^{b}		0.88^{a}	0.76^{b}	0.71^{b}	

* Mean of three replications

In the table figures followed by same letter do not differ significantly according to DMRT

fresh weight recorded at 30°C (11.03g). Among the isolates, bottle gourd isolate recorded maximum fresh weight (13.16g) as a result of total effect of temperature. It was followed by the isolate from snake gourd (12.61g) and the latter was on par with bottle gourd. The interaction effect of temperature and isolates was also significant. The isolate from bottle gourd recorded maximum fresh weight of 16.83g at 25°C, which was on par with from snake gourd at 25°C (14.70g) and bottle gourd at 30°C(13.67g). The lowest fresh weight was recorded by ivy gourd isolate at 35°C (5.55g).

From the results furnished in Table 18 it was revealed that among the three different temperatures tested maximum dry weight was recorded at 25°C (0.88g). The total effect of temperature of different isolates resulted maximum dry weight of 0.91g from snake gourd isolate, from bitter gourd (0.82g), from bottle gourd (0.81g) and from pumpkin (0.81g). Isolate from snake gourd at 25°C produced maximum dry weight (1.12g), which was on par with the isolate from pumpkin at 25°C (1.01g), from bottle gourd at 25°C (0.96g) and 35°C (0.82g), from snake gourd at 30°C (0.95g), from ash gourd at 35°C (0.90g), bitter gourd at 25°C (0.88g) and 30°C (0.87g) and from ridge gourd at 25°C (0.85g). Minimum dry weight was recorded by the isolate from ridge gourd at 35°C (0.54g).

4.5.1.2 Isolates of Alternaria from Elanad

From data given in Table 19, it was found that among the temperatures maximum fresh weight was recorded at 30°C (14.80g) and among the different isolates, the isolate from ridge gourd recorded maximum fresh weight (17.33g), which was on par with bottle gourd (17.29g). The interaction effect of temperature and isolates was also significant. Ridge gourd at 30°C recorded maximum fresh weight (19.67g), which was on par with bottle gourd at 30°C (18.52g) and 25°C (18.16g) and ridge gourd at 25°C (18.08g). Fresh weight was minimum for snake gourd at 35°C (6.45g).

The data (Table 19) revealed that the isolate from ivy gourd recorded maximum dry weight (1.40g) which was on par with bitter gourd (1.12g) and ash gourd

Table 19. Effect of temperature on fresh and dry weight of isolates of *Alternaria* from Elanad

Sl. No.	Isolates	*Fresh weight (g)				*Dry weight (g)			
		25°C	30°C	35°C	Mean	25°C	30°C	35°C	Mean
1	Ash gourd	14.56^{bc}	14.69^{bc}	11.46^{de}	13.57^{b}	1.03^{bcd}	1.09^{bcd}	1.12^{bcd}	1.08^{ab}
2	Snake gourd	9.63^{ef}	12.19^{cde}	6.45^{g}	9.43^{c}	0.92^{bcd}	0.96^{bcd}	1.00^{bcd}	0.96^{b}
3	Ridge gourd	18.08^{a}	19.67^{a}	14.25^{bc}	17.33^{a}	0.99^{bcd}	0.93^{bcd}	0.80^{cd}	0.91^{b}
4	Bottle gourd	18.16^{a}	18.52^{a}	15.19^{b}	17.29^{a}	1.17^{bc}	0.92^{bcd}	0.74^{d}	0.94^{b}
5	Bitter gourd	8.33^{fg}	10.60^{def}	9.50^{ef}	9.48^{c}	1.23^{b}	1.10^{bcd}	1.03^{bcd}	1.12^{ab}
6	Ivy gourd	12.06^{cde}	13.12^{bcd}	12.21^{cde}	12.46^{b}	1.10^{bcd}	1.06^{bcd}	2.03^{a}	1.40^{a}
7	Mean	13.47^{b}	14.80^{a}	11.51^{c}		1.07^{a}	1.01^{a}	1.12^{a}	

* Mean of three replications

In the table figures followed by same letter do not differ significantly according to DMRT

(1.08g) and no significant difference in statistical analysis was observed between the three temperatures to influence the dry weight of the isolates. The interaction effect of isolates and temperature showed that ivy gourd at 35°C recorded maximum dry weight (2.03g) and followed by bitter gourd at 25°C(1.23g). A minimum dry weight was recorded for bottle gourd at 35°C (0.74g).

4.5.2 Effect of p^H on the fresh and dry weight of isolates of *Alternaria*

The fresh and dry weight of mycelial growth of different isolates of *Alternaria* was recorded in three different p^H conditions of 6, 7 and 8. Statistical analysis of the data revealed significant difference among the treatments and different isolates.

4.5.2.1 Isolates of Alternaria from Vellanikkara

From the results showed in Table 20, it was found that among the different p^H levels tested, in the p^H 6, the isolates produced maximum fresh weight of 9.86g, which was on par with the fresh weight at p^H 7(9.56g). In general snake gourd recorded the maximum fresh weight (12.14g) among the different isolates and was also on par with 11.77g of ash gourd. Interaction effect of isolates and p^H showed that, snake gourd at p^H 7 recorded maximum fresh weight of 18.80g and it was statistically superior among the isolates in recording maximum fresh weight. The isolate from bottle gourd recorded a minimum fresh weight (6.21g) at p^H 7.

From the data given in Table 20, it was found that among the different p^H levels, p^H 6 recorded maximum dry weight of 0.69g and no significant difference in statistical analysis was noticed among the different isolates in producing dry weight. Interaction effect of isolates and p^H was also significant. It showed that, pumpkin isolate at p^H 6 recorded maximum dry weight (0.94g), which was statistically on par with bottle gourd at p^H 6 (0.82g), snake gourd at p^H 6 (0.74g) and bitter gourd at p^H 6 (0.73). Snake gourd at p^H 8 produced minimum dry weight of 0.30g.

Table 20. Effect of p^{H} on fresh and dry weight of isolates of *Alternaria* from Vellanikkara

Sl. No.	Isolates	*Fresh weight (g)				*Dry weight (g)			
		p^{H} 6	p^{H} 7	p^{H} 8	Mean	p^{H} 6	p^{H} 7	p^{H} 8	Mean
1	Ash gourd	14.87^{b}	9.41^{cdef}	11.02^{cd}	11.77^{a}	0.50^{cdef}	0.52^{bcdef}	0.42^{def}	0.48^{a}
2	Snake gourd	9.92^{cde}	18.80^{a}	7.69^{efg}	12.14^{a}	0.74^{abc}	0.43^{cdef}	0.30^{f}	0.49^{a}
3	Ridge gourd	11.10^{c}	7.91^{efg}	8.00^{defg}	9.00^{b}	0.53^{bcdef}	0.45^{cdef}	0.50^{cdef}	0.49^{a}
4	Bottle gourd	8.53^{cdefg}	6.21^{g}	8.69^{cdefg}	7.81^{bc}	0.82^{ab}	0.42^{def}	0.51^{bcdef}	0.58^{a}
5	Bitter gourd	9.30^{cdef}	9.51^{cdef}	8.65^{cdefg}	9.15^{b}	0.73^{abcd}	0.53^{bcdef}	0.53^{bcdef}	0.60^{a}
6	Ivy gourd	7.13^{efg}	6.73^{fg}	7.83^{efg}	7.23^{c}	0.61^{bcdef}	0.43^{cdef}	0.48^{cdef}	0.51^{a}
7	Pumpkin	8.19^{cdefg}	8.34^{cdefg}	7.86^{efg}	8.13^{bc}	0.94^{a}	0.37^{ef}	0.62^{bcde}	0.65^{a}
	Mean	9.86^{a}	9.56^{a}	8.54^{b}		0.69^{a}	0.45^{b}	0.48^{b}	

* Mean of three replications

In the table figures followed by same letter do not differ significantly according to DMRT

4.5.2.2 Isolates of Alternaria from Elanad

From the data presented in table 21, it was observed that among the different p^H levels tested, p^H 6 was found to be producing maximum fresh weight (12.48g), which was also on par with p^H 7(12.43g). In general ivy gourd recorded a maximum fresh weight of 13.40g, which was on par with bitter gourd (12.21g). The isolate from bitter gourd at p^H 7 was found to producing maximum fresh weight of 14.98g which was on par with ivy gourd at p^H 7 (14.97g) and p^H 6 (13.53g), bitter gourd at p^H 6 (13.69g), bottle gourd at p^H 7 (12.63g) and ash gourd at p^H 6 (12.57g). Minimum fresh weight was recorded by bitter gourd (7.94g) at p^H 8, which was on par with snake gourd at p^H 8 (8.19g).

Observations on dry weight (Table 21) showed that among the different isolates, the isolate from bitter gourd recorded maximum dry weight (1.47g) and among the different p^H levels tested, p^H 7 recorded maximum dry weight (1.30g). Interaction effect of isolates and p^H revealed that bitter gourd isolate at p^H 7 recorded maximum dry weight (2.64g) and a minimum dry weight of 0.39g was recorded by ash gourd at p^H 8.

4.5.3 Effect of light on the fresh and dry weight of isolates of *Alternaria*

The effect of light on the fresh and dry weight of isolates of *Alternaria* was recorded at full light, full dark and alternate light and dark. The data showed significant difference among the treatments.

4.5.3.1 Isolates of Alternaria from Vellanikkara

The results presented in Table 22 showed that, among the isolates, snake gourd recorded maximum fresh weight (12.58g) and the light treatments tested full light recorded maximum fresh weight (10.44), which was on par with alternate light and dark (9.82g). Maximum fresh weight was recorded by snake gourd isolate at alternate light and dark (13.64g) which was on par with snake gourd at full dark (13.38g) and full light (10.74g), bitter gourd at full light (13.08), bottle gourd at full light (12.53g), pumpkin at

Table 21. Effect of p^H on fresh and dry weight of isolates of *Alternaria* from Elanad

Sl. No.	Isolates	*Fresh weight (g)				*Dry weight (g)			
		p^H 6	p^H 7	p^H 8	Mean	p^H 6	p^H 7	p^H 8	Mean
1	Ash gourd	12.57^{abcd}	11.02^{bcde}	9.95^{def}	11.18^{bc}	0.72^{cde}	1.09^{c}	0.39^{e}	0.73^{c}
2	Snake gourd	11.82^{bcd}	12.21^{bcd}	8.19^{f}	10.74^{bc}	0.72^{cde}	0.56^{de}	0.61^{cde}	0.63^{c}
3	Ridge gourd	11.10^{bcde}	8.74^{ef}	11.24^{bcde}	10.69^{c}	0.62^{cde}	0.80^{cde}	0.82^{cde}	0.75^{c}
4	Bottle gourd	11.15^{bcde}	12.63^{abcd}	10.88^{cde}	11.55^{bc}	0.83^{cde}	0.51^{e}	0.49^{g}	0.61^{c}
5	Bitter gourd	13.69^{ab}	14.98^{a}	7.94^{f}	12.21^{ab}	1.04^{cd}	2.64^{a}	0.73^{cde}	1.47^{a}
6	Ivy gourd	13.53^{abc}	14.97^{a}	11.70^{bcd}	13.40^{a}	0.64^{cde}	2.23^{b}	0.57^{de}	1.14^{b}
7	Mean	12.48^{a}	12.43^{a}	9.98^{b}		0.76^{b}	1.30^{a}	0.60^{b}	

* Mean of three replications

In the table figures followed by same letter do not differ significantly according to DMRT

Table 22. Effect of light on fresh and dry weight of isolates of *Alternaria* from Vellanikkara

Sl. No.	Isolates	*Fresh weight (g)				*Dry weight (g)			
		Full dark	Light + Dark	Full light	Mean	Full dark	Light + Dasrk	Full light	Mean
1	Ash gourd	7.98^{de}	9.33^{bcde}	9.11^{cde}	8.81^{bc}	0.58^{efghi}	0.95^{cd}	0.86^{de}	0.79^{b}
2	Snake gourd	13.38^{a}	13.64^{a}	10.74^{abcde}	12.58^{a}	1.24^{bc}	1.44^{b}	1.76^{a}	1.48^{a}
3	Ridge gourd	3.96^{f}	9.22^{cde}	9.97^{abcde}	7.72^{cd}	0.26^{i}	0.67^{defgh}	0.76^{def}	0.56^{c}
4	Bottle gourd	7.10^{def}	9.14^{cde}	12.53^{abc}	9.59^{bc}	0.26^{i}	0.37^{ghi}	0.72^{defg}	0.45^{cd}
5	Bitter gourd	8.41^{de}	9.41^{bcde}	13.08^{ab}	10.30^{b}	0.32^{hi}	0.37^{ghi}	0.31^{hi}	0.33^{d}
6	Ivy gourd	3.90^{f}	9.31^{bcde}	6.84^{ef}	6.68^{d}	0.28^{i}	0.50^{fghi}	0.48^{fghi}	0.42^{cd}
7	Pumpkin	7.47^{def}	8.66^{cde}	10.84^{abcd}	8.99^{bc}	0.35^{hi}	0.50^{fghi}	0.78^{def}	0.54^{c}
	Mean	7.46^{b}	9.82^{a}	10.44^{a}		0.47^{b}	0.68^{a}	0.81^{a}	

* Mean of three replications

In the table figures followed by same letter do not differ significantly according to DMRT

full light (10.84g) and ridge gourd at full light (9.97g).And minimum fresh weight was recorded by the isolate ivy gourd at full dark (3.90g).

The results in Table 22 showed that, the isolate from snake gourd recorded maximum dry weight of 1.48g among the different isolates and full light recorded a maximum dry weight of 0.81g, which was on par with alternate light and dark (0.68g). The interaction effect of isolates and light showed that snake gourd isolate at full light produced maximum dry weight (1.76g) and it was statistically superior among the different isolates tested. The minimum dry weight was recorded by the isolates ridge gourd and bottle gourd at full dark (0.26g).

4.5.3.2 Isolates of Alternaria from Elanad

From the data presented in Table 23, it was found that among the different light treatments tested full dark recorded maximum fresh weight (14.62g), which was on par with alternate light and dark (14.34g). Generally, bottle gourd isolate recorded maximum fresh weight (17.54g) among the different isolates, which was on par with ridge gourd (16.47g). The interaction effect showed that, the isolate from bottle gourd at alternate light and dark produced maximum fresh weight (18.22g) which was on par with bottle gourd at full dark (17.96g) and full light (16.43g), ridge gourd at alternate light and dark (17.20g), full dark (16.74g) and full light (15.48g), snake gourd at full dark (16.83g) and ivy gourd at full light (15.87g). A minimum fresh weight of 9.00g was recorded by ash gourd isolate at full light.

The observations on dry weight (Table 23) showed that among the different isolates tested ivy gourd isolate (1.83g) was statistically superior to all other isolates in producing dry weight and among the different light treatments full light recorded maximum dry weight (1.45g). The interaction effect of light and isolates showed that ivy gourd at full light recorded maximum dry weight of 3.03g and it was statistically superior to all other isolates. A minimum dry weight was recorded by snake gourd at full light (0.69g).

Table 23. Effect of light on fresh and dry weight of isolates of *Alternaria* from Elanad

Sl. No.	Isolates	*Fresh weight (g)				*Dry weight (g)			
		Full dark	Light + Dark	Full light	Mean	Full dark	Light + Dark	Full light	Mean
1	Ash gourd	12.92^{defg}	13.13^{cdefg}	9.00^{h}	11.68^{c}	1.09^{bc}	1.18^{bc}	1.14^{bc}	1.34^{b}
2	Snake gourd	16.83^{abc}	12.85^{defg}	10.01^{fgh}	13.23^{bc}	1.31^{b}	1.25^{bc}	0.69^{c}	1.08^{b}
3	Ridge gourd	16.74^{abc}	17.20^{ab}	15.48^{abcde}	16.47^{a}	1.23^{bc}	1.07^{bc}	1.32^{b}	1.20^{b}
4	Bottle gourd	17.96^{a}	18.22^{a}	16.43^{abcd}	17.54^{a}	1.28^{b}	1.12^{bc}	1.16^{bc}	1.19^{b}
5	Bitter gourd	9.66^{gf}	13.04^{cdefg}	12.22^{efgh}	11.64^{c}	1.08^{bc}	1.32^{b}	1.34^{b}	1.25^{b}
6	Ivy gourd	13.60^{bcdef}	11.61^{fgh}	15.87^{abcde}	13.70^{b}	1.25^{bc}	1.20^{bc}	3.03^{a}	1.83^{a}
7	Mean	14.62^{a}	14.34^{ab}	$13.1\ 7^{b}$		1.21^{b}	1.19^{b}	1.45^{a}	

* Mean of three replications

In the table figures followed by same letter do not differ significantly according to DMRT

4.5.4 Effect of carbon sources on the fresh and dry weight of isolates of *Alternaria*

The fresh weight and dry weight of mycelial growth of different isolates of *Alternaria* spp. were recorded by using three different carbon sources *viz.*, sucrose, starch and lactose. The data showed significant difference among the treatments.

4.5.4.1 Isolates of Alternaria from Vellanikkara

From the Table 24, it was revealed that, among the different isolates snake gourd isolate recorded maximum fresh weight of 13.03g, which was statistically on par with ridge gourd (11.96g). Among the different carbon sources tested, starch produced maximum fresh weight (12.69g) of the isolates. Interaction between carbon sources and isolates showed that the isolate of ridge gourd grown on medium with starch as carbon source recorded maximum fresh weight of (15.79g). It was statistically on par with snake gourd (14.97g), pumpkin (13.83g), bottle gourd (13.81g), bitter gourd (13.56g) and ash gourd (13.29g) on starch and snake gourd (12.99g) and ridge gourd (12.64g) on sucrose. The minimum fresh weight was recorded by ivy gourd in starch (5.49g).

The results presented in Table 24 showed that, starch produced maximum dry weight (1.13g) among the different carbon sources, which was on par with sucrose (1.12g). Among the different isolates, bottle gourd recorded maximum dry weight (1.46g), which was statistically superior to all other carbon sources tested. The interaction effect showed that, among the different isolates, bottle gourd recorded maximum dry weight in lactose as carbon source (1.80g), which was on par with ridge gourd in sucrose as carbon source (1.55g). Minimum dry weight was recorded by pumpkin isolate in lactose medium (0.67g).

4.5.4.2 Isolates of Alternaria from Elanad

The data presented in Table 25 revealed that, bitter gourd isolate produced maximum fresh weight (18.46g) and it was statistically superior to all other isolates tested. Among the different carbon sources, starch produced maximum fresh weight

Table 24. Effect of carbon sources on fresh and dry weight of isolates of *Alternaria* from Vellanikkara

Sl. No.	Isolates	*Fresh weight (g)				*Dry weight (g)			
		Starch	Lactose	Sucrose	Mean	Starch	Lactose	Sucrose	Mean
1	Ash gourd	13.29^{abc}	10.59^{bcdef}	7.85^{efg}	10.58^{b}	0.81^{fghi}	0.97^{efghi}	1.09^{defg}	0.96^{cde}
2	Snake gourd	14.97^{a}	11.12^{bcde}	12.99^{abc}	13.03^{a}	0.75^{hi}	0.83^{fghi}	0.95^{efgh}I	0.84^{e}
3	Ridge gourd	15.79^{a}	7.44^{fg}	12.64^{abcd}	11.96^{ab}	1.20^{cde}	0.80^{ghi}	1.55^{ab}	1.18^{b}
4	Bottle gourd	13.81^{ab}	8.50^{efg}	10.82^{bcdef}	11.05^{b}	1.42^{bc}	1.80^{a}	1.14^{cdef}	1.46^{a}
5	Bitter gourd	13.56^{abc}	8.20^{efg}	9.16^{def}	10.30^{b}	1.38^{bcd}	1.01^{efgh}	0.98^{efgh}I	1.12^{bc}
6	Ivy gourd	5.49^{g}	7.76^{efg}	11.00^{bcdef}	8.08^{c}	0.91^{efghi}	0.83^{fghi}	1.09^{defg}	0.94^{de}
7	Pumpkin	13.83^{ab}	10.17^{cdef}	9.36^{def}	11.12^{b}	1.43^{bc}	0.67^{i}	1.06^{efgh}	1.05^{bcd}
	Mean	12.69^{a}	9.11^{c}	10.55^{b}		1.13^{a}	0.99^{b}	1.12^{a}	

* Mean of three replications

In the table figures followed by same letter do not differ significantly according to DMRT

Table 25. Effect of carbon sources on fresh and dry weight of isolates of *Alternaria* from Elanad

Sl. No.	Isolates	*Fresh weight (g)				*Dry weight (g)			
		Starch	Lactose	Sucrose	Mean	Starch	Lactose	Sucrose	Mean
1	Ash gourd	16.68^{cde}	11.17^{gh}	10.65^{gh}	12.83^{cd}	1.21^{bc}	1.24^{bc}	1.40^{b}	1.28^{b}
2	Snake gourd	14.49^{ef}	10.41^{h}	11.63^{gh}	12.18^{d}	0.86^{def}	1.17^{bcd}	1.10^{bcde}	1.04^{c}
3	Ridge gourd	21.56^{a}	13.52^{fg}	10.38^{h}	15.16^{b}	0.64^{f}	1.11^{bcde}	0.98^{cde}	0.91^{c}
4	Bottle gourd	19.38^{abc}	12.09^{fgh}	9.70^{h}	13.72^{bc}	0.82^{def}	1.16^{bcd}	1.15^{bcd}	1.04^{c}
5	Bitter gourd	20.59^{ab}	16.25^{de}	18.54^{bcd}	18.46^{a}	0.94^{cdef}	2.56^{a}	1.13^{bcd}	1.54^{a}
6	Ivy gourd	18.93^{abcd}	10.77^{gh}	11.97^{fgh}	13.89^{bc}	0.78^{e}f	1.36^{b}	1.23^{bc}	1.13^{bc}
7	Mean	18.61^{a}	12.37^{b}	12.14^{b}		0.88^{c}	1.44^{a}	1.17^{b}	

* Mean of three replications

In the table figures followed by same letter do not differ significantly according to DMRT

(18.61g) of the isolates. The interaction effect of carbon sources and isolates showed that maximum fresh weight was recorded by ridge gourd isolate (21.56g) grown on starch medium and it was statistically on par with bitter gourd (20.59g), bottle gourd (19.38g), and ivy gourd (18.93g) in starch. A minimum fresh weight was recorded by the snake gourd isolate (10.41g) grown on lactose medium, which was on par with ridge gourd (10.38g) and bottle gourd (9.70g) grown on sucrose medium.

From the results furnished in Table 25, it was shown that the bitter gourd isolate recorded a maximum dry weight of 1.54g and lactose (1.44g) containing media supported the maximum dry weight of the isolates. The interaction effect revealed that the isolate bitter gourd recorded maximum dry weight (2.56g) on lactose medium, which was statistically superior to all other isolates. A minimum dry weight was recorded by ridge gourd isolate (0.64g) grown on starch medium.

4.5.5 Effect of nitrogen sources on the fresh and dry weight of isolates of *Alternaria*

The effect of different nitrogen sources *viz.*, KNO_3, $MgNO_3$ and $NaNO_3$ on fresh weight and dry weight of mycelial growth of different isolates of *Alternaria* were recorded in Table 26 and significant difference was noticed among the treatments.

4.5.5.1 Isolates of Alternaria from Vellanikkara

From the Table 26 it is noticed that among the different isolates tested snake gourd produced maximum fresh weight (12.82g), which was statistically superior to all other isolates and the nitrogen source KNO_3 (10.19g) recorded maximum fresh weight, which was on par with $MgNO_3$ (9.60g). The interaction effect of isolates and nitrogen sources was also significant and the isolate snake gourd grown on KNO_3 as the nitrogen source recorded maximum fresh weight of 14.68g which was on par with bottle gourd on KNO_3 (12.61g) as nitrogen source. A minimum fresh weight was recorded by ivy gourd isolate (4.83g) on the media containing the same nitrogen source.

Table 26. Effect of nitrogen sources on fresh and dry weight of isolates of *Alternaria* from Vellanikkara

Sl. No.	Isolates	*Fresh weight (g)				*Dry weight (g)			
		KNO_3	$MgNO_3$	$NaNO_3$	Mean	KNO_3	$MgNO_3$	$NaNO_3$	Mean
1	Ash gourd	7.62^{fghi}	7.96^{efghi}	6.70^{hij}	7.43^{cd}	0.39^{g}	0.75^{bcdef}	0.59^{defg}	0.57^{c}
2	Snake gourd	14.68^{a}	11.69^{bc}	12.08^{bc}	12.82^{a}	1.16^{a}	0.87^{abcd}	1.00^{ab}	1.01^{a}
3	Ridge gourd	10.73^{bcd}	9.89^{cdef}	10.63^{bcd}	10.42^{b}	0.86^{abcde}	1.08^{a}	0.51^{fg}	0.81^{b}
4	Bottle gourd	12.61^{ab}	9.14^{defg}	8.43^{defgh}	10.10^{b}	0.39^{g}	0.90^{abcd}	0.60^{cdefg}	0.63^{bc}
5	Bitter gourd	8.72^{defgh}	8.95^{defgh}	6.91^{ghij}	8.20^{c}	0.91^{abc}	0.54^{efg}	0.58^{defg}	0.67^{bc}
6	Ivy gourd	4.83^{j}	9.26^{defg}	5.95^{ij}	6.68^{d}	0.62^{cdefg}	0.59^{cdefg}	0.63^{cdefg}	0.62^{bc}
7	Pumpkin	12.14^{bc}	10.30^{bcde}	9.80^{cdef}	10.75^{b}	0.67^{cdefg}	0.92^{abc}	0.66^{cdefg}	0.75^{bc}
	Mean	10.19^{a}	9.60^{a}	8.64^{b}		0.71^{ab}	0.81^{a}	0.65^{b}	

* Mean of three replications

In the table figures followed by same letter do not differ significantly according to DMRT

The results furnished in Table 26 showed that, among the different isolates snake gourd produced maximum dry weight of 1.01g and among the different nitrogen sources $MgNO_3$ (0.81g) recorded maximum dry weight, which was on par with KNO_3 (0.71g).The interaction effect showed that the snake gourd isolate grown on KNO_3 recorded maximum dry weight of 1.16g which was on par with snake gourd on $NaNO_3$ (1.00g) and $MgNO_3$ (0.87g), Pumpkin on $MgNO_3$ (0.92g), bitter gourd on KNO_3 (0.91g) bottle gourd on $MgNO_3$ (0.90g) and ridge gourd on $MgNO_3$ (1.08g) and KNO_3 (0.86g). Minimum dry weight was recorded for bottle gourd (0.39g) and ash gourd (0.39g).

4.5.5.2 Isolates of Alternaria from Elanad

The results (Table 27) revealed that among the different isolates bottle gourd recorded maximum dry weight (17.55g), which was on par with ridge gourd (17.08g). No significant difference in statistical analysis was observed between the three nitrogen sources to influence the fresh weight of the isolates. Observation on interaction effect of isolates and nitrogen sources showed that ridge gourd isolate (18.43g) recorded maximum fresh weight on $NaNO_3$ containing media and which was on par with bottle gourd (18.21g) and ridge gourd (18.11g) on KNO_3, bottle gourd on $MgNO_3$ (17.67g) and NaNO3 (16.78g) and ridge gourd on $MgNO_3$ (14.70g). The minimum fresh weight was recorded by snake gourd on $MgNO_3$ (9.25g), which was on par with snake gourd on $NaNO_3$ (9.33g).

The observations on (Table 27) on dry weight showed that $NaNO_3$ (1.20g) recorded maximum dry weight which was on par with $MgNO_3$ (1.12g) and no significant difference in statistical analysis was observed between the different isolates. Interaction effect of nitrogen sources and isolates revealed that snake gourd isolate produced maximum dry weight of 1.72 g on $NaNO_3$, which was on par with bitter gourd on Mg NO_3 (1.72g) and ash gourd on $NaNO_3$ (1.61g). The minimum dry weight was recorded by ash gourd on $MgNO_3$ (0.81g), which was on par with all other isolates.

Table 27. Effect of nitrogen sources on fresh and dry weight of isolates of *Alternaria* from Elanad

Sl. No.	Isolates	*Fresh weight of mycelial growth (g)				*Dry weight of mycelial growth (g)			
		KNO_3	$MgNO_3$	$NaNO_3$	Mean	KNO_3	$MgNO_3$	$NaNO_3$	Mean
1	Ash gourd	10.39^{de}	12.66^{cde}	9.17^{e}	10.74^{bc}	1.02^{b}	0.81^{b}	1.61^{a}	1.15^{a}
2	Snake gourd	10.29^{de}	9.25^{e}	9.33^{e}	9.62^{c}	0.87^{b}	0.92^{b}	1.72^{a}	1.17^{a}
3	Ridge gourd	18.11^{a}	14.70^{abc}	18.43^{a}	17.08^{a}	1.08^{b}	1.16^{b}	1.18^{b}	1.14^{a}
4	Bottle gourd	18.21^{a}	17.67^{a}	16.78^{ab}	17.55^{a}	1.19^{b}	1.15^{b}	0.82^{b}	1.08^{a}
5	Bitter gourd	13.66^{bcd}	12.43^{cde}	10.56^{de}	12.22 ^{b}	1.06^{b}	1.72^{a}	0.93^{b}	1.24^{a}
6	Ivy gourd	11.34^{cde}	10.42^{de}	10.98^{cde}	10.91^{bc}	0.94^{b}	0.95^{b}	0.95^{b}	0.95^{a}
7	Mean	13.67^{a}	12.85^{a}	12.54^{a}		1.06^{b}	1.12A^{b}	1.20^{a}	

* Mean of three replications

In the table figures followed by same letter do not differ significantly according to DMRT

4.6 CONIDIAL GERMINATION OF DIFFERENT ISOLATES OF *ALTERNARIA*

Conidial suspension of *Alternaria* used in the study was prepared separately, incubated and the percent germination was calculated at hourly intervals for a period of maximum conidial germination. The results are presented in Table 28.

It was found that conidia of all the isolates started germination after three hours of incubation and after 10h all of them showed cent per cent germination. It was also noticed that in majority of cases all the cells of conidium germinated by producing germ tube. However appressoria formations were not observed (Plate 9A).

4.7 COMPATIBILITY AMONG DIFFERENT ISOLATES OF *ALTERNARIA*

Compatibility among different isolates of *Alternaria* was studied following the dual culture technique by inoculating isolates in different combinations.

All isolates of the same and different locations were found to be compatible with each other. The microscopic observation revealed free intermingling of hyphae of the isolates and abundant production of conidia at the point of contact.

4.8 HISTOPATHOLOGICAL STUDIES

For studying histopathological changes due to *Alternaria* infection on cucurbitaceous vegetables *viz.*, ash gourd, snake gourd, ridge gourd, bottle gourd, bitter gourd, ivy gourd and pumpkin, thin hand sections of leaf blight affected areas were taken and stained with Saffranin and observed under the microscope.

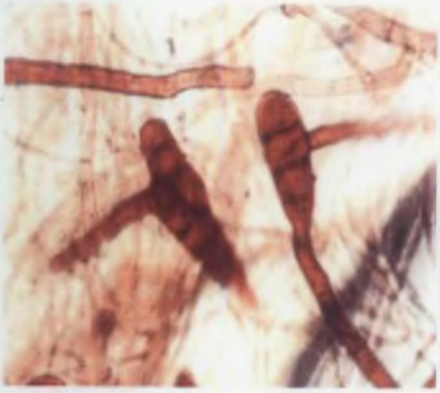
In general due to infection epidermal cells were found damaged. The fungal hyphae and conidiophores were seen emerging through the damaged epidermis (Plate 9B). Cells in the palisade tissues were found distorted. Disruptions in the distribution pattern of chloroplast and disintegration of chlorophyll grains were observed. In certain infected portions of leaves, the epidermis and cells in the palisade tissues were found shrunken and converged to a central point. During advanced stage, complete necrosis and

Table 28. Per cent germination of conidia of different isolates of *Alternaria*

Sl. No.	Isolates	Locations	Per cent germination (Hours after incubation)									
			1	2	3	4	5	6	7	8	9	10
1	Ash gourd	VKA	-	-	4.38	16.21	24.34	48.61	53.33	72.67	95.0	100
		ELA	-	-	13.11	20.18	38.27	50.33	61.40	84.22	96.31	100
	Snake gourd	VKA	-	-	16.67	26.68	50.80	60.51	83.30	90.00	96.67	100
		ELA	-	-	11.1	25.92	29.63	40.74	55.56	74.07	92.59	100
3	Ridge gourd	VKA	-	-	6.30	17.81	35.33	72.80	79.21	88.33	96.50	100
		ELA	-	-	7.50	13.3	33.10	53.5	60.00	73.3	86.00	100
4	Bottle gourd	VKA	-	-	16.10	22.58	35.47	48.39	74.19	88.00	93.55	100
		ELA	-	-	13.67	26.61	33.68	50.00	70.31	73.88	86.90	100
5	Bitter gourd	VKA	-	-	11.10	27.78	38.89	55.56	61.1	83.3	94.4	100
		ELA	-	-	10.71	25.00	33.31	39.29	67.85	78.57	89.29	100
6	Ivy gourd	VKA	-	-	17.86	28.57	35.71	42.86	71.43	78.57	89.30	100
		ELA	-	-	6.3	18.00	33.3	71.5	78.5	86.3	92.00	100
7	Pumpkin	VKA	-	-	13.58	25.67	32.31	45.75	78.33	82.61	93.33	100

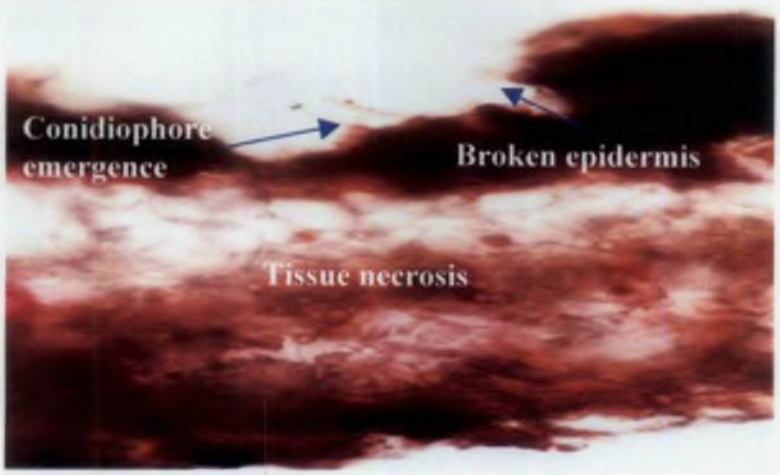
Plate 9A. Conidial germination

(400 X)



(1000 X)

Plate 9B. Histopathology



death of whole cells including upper and lower epidermis were noticed due to the infection.

4.9 HOST RANGE

For studying the host range of *Alternaria* isolates used in the present investigation, pure cultures of each of the isolates were cross inoculated on different cucurbits and also on other vegetables *viz.*, tomato, chilli, brinjal, amaranthus, cowpea and bhindi. It was observed that all the host plants tested took up infection when inoculated with different isolates.

On the inoculated leaves of host plants tested, the symptoms appeared five to seven days after inoculation as yellow discolouration on the inoculated portion. Later the yellow discolouration changed to dark brown in colour and enlarged in size with characteristic yellow halo. In bhindi, the lesion developed as small irregular blighted areas which enlarged in size and cover most of the leaf area with in four to five days (Plate 10).

4.10 EFFECT OF TOXIC METABOLITES OF *ALTERNARIA* ON PATHOGENESIS

In order to study the effect of toxic metabolites of different isolates of *Alternaria* on the causation of leaf blight disease, the leaves of different cucurbitaceous vegetables raised in polybags were spot inoculated separately with the exotoxin and endotoxin extracted from the respective isolates. Cross inoculations of toxic metabolites on cucurbits and other vegetables were also done. It was observed that exotoxins and endotoxins produced by different isolates of *Alternaria* isolated from the two locations produced symptom on all the cucurbitaceous vegetables and other vegetables tested (Plate 11). However visible symptoms were first noticed on leaves inoculated with exotoxin. Exotoxin inoculated leaves first showed yellowing on inoculated area then followed by brown lesions after five days of inoculation. In the case of endotoxin inoculated plants, characteristic marginal leaf blight symptoms were observed six days after inoculation. Symptom developed as yellowing on the inoculated portion and leaf blightening from the margins of the leaves.

Plate 10. Host range

a. Tomato

b. Chilli

c. Brinjal

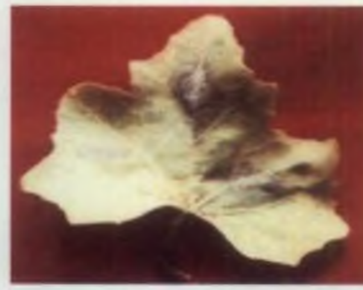
d. Amaranthus

e. Cowpea

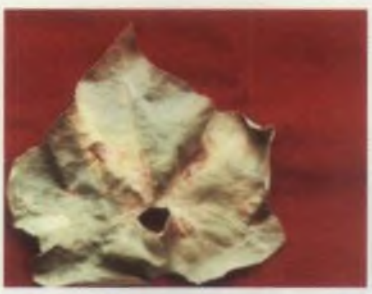
f. Bhindi

Plate 11. Effect of toxin on pathogenesis of leaf blight diseases of cucurbitaceous vegetables

a. Ash gourd



b. Snake gourd



c. Ridge gourd

d. Bottle gourd

e. Bitter gourd

f. Coccinia

g. Pumpkin

Discussion

5. DISCUSSION

Cucurbitaceous vegetables, consisting of thirty species of cultivated one, possess an important role in human diet. They are grown for their edible fruits, which are consumed either fresh or after cooking. Cucurbits are known to be affected by a number of diseases. Among them *Alternaria* leaf blight is the one which is known to cause considerable crop losses. A perusal of literature revealed that not much work has been carried out on the various aspects of this disease. However from Rajasthan, *Alternaria* blight of watermelon inflicting serious losses was reported by Khandelwal and Prasada (1970). Chahal *et al.* (1970) recorded *Alternaria* leaf spot disease on various cucurbits *viz.*, muskmelon, cucumber, bottle gourd and watermelon for the first time from Ludhiana and identified the pathogen as *Alternaria cucumerina*. Later, many workers from different places of India reported the occurrence of *A. alternata* (Narain *et al.*, 1985; Bedi *et al.*, 1993; Davis, 2003) and *A. tenuissima* (Kumar *et al.*, 2001) as the causal agent of leaf blight diseases of cucurbitaceous vegetables.

Since, cucurbitaceous vegetables are known to be infected either by one or more species of *Alternaria*, it is imperative to have a detailed investigation on the existence of variability among them. Reports from elsewhere were also suggestive of existence of variability among isolates of *Alternaria* infecting cucurbits. Thus the present investigation has been carried out to locate variability if any exist among the isolates of *Alternaria* causing leaf blight in cucurbitaceous vegetables of selected fields of Thrissur district.

As the first part of the study a survey was conducted to find out the occurrence of leaf blight infections in two locations of Thrissur district. It was observed that ash gourd, snake gourd, ridge gourd, bottle gourd, bitter gourd, ivy gourd and pumpkin were severely infected by leaf blight disease. Two types of leaf blights were noticed, one with characteristic concentric zonations and other with out concentric zonations. Isolation from the collected specimens yielded fungal growth resembling *Alternaria* sp. on PDA medium. Seven culture of *Alternaria* were isolated from

Vellanikkara and six from Elanad area. The pathogenicity of these isolates was proved by artificial inoculation on its respective cucurbitaceous hosts under *in vitro* and *in vivo* conditions.

In the disease cycle, symptom expression is the most important part in the chain of events and thus a better understanding of the symptomatology of a disease will be helpful in the correct identification of a disease in a given situation. Further, symptoms produced by the same pathogen may vary depending upon the host, virulence of the pathogen and also by the environmental conditions. Therefore a detailed study on the symptomatology of leaf blight disease produced in all the cucurbitaceous vegetables were carried out under natural condition to understand whether there is any variation in symptom expression.

In general it was noticed that leaf blight in cucurbits was initiated from the margins as small, irregular brown lesion. Later these lesions enlarged in size resulting in blightening of leaves. Invariably the lesions were surrounded by a yellow halo. Concentric zonations on the infected area were present in blightening caused by ash gourd, bitter gourd and ivy gourd. However concentric zonations were not found on infected portions of leaves of snake gourd, ridge gourd, bottle gourd and pumpkin. Hence the only variation in symptom expression was with respect to the presence or absence of concentric zonations on the blightened area. Even though *A. alternata* and *A. tenuissima* were reported from different host plants, the different species did not cause much variation in symptom expression. The symptomatology of *Alternaria* blight in cucurbits was studied by many workers (Chahal *et al.*, 1970; Vakalounakis and Malathrakis, 1988). They also reported almost same symptom expression as that observed during the present study. Bhargava and Singh (1985) and Davis (2003) also reported the presence of concentric zonations in *Alternaria* infection on watermelon and ivy gourd respectively.

The cultural and morphological characters of fungal pathogen are considered as important criteria for their correct identification. However within a species itself variability may exist with respect to same characters which may directly contribute to variability. So, for the identification of different isolates of *Alternaria* from various cucurbitaceous host plants, and also to know variability if any among them, the cultural and morphological characters of isolates were studied. All the isolates of pathogen produced thick and velvety colony with brownish grey to dark brown aerial mycelium. The isolates from ash gourd, ridge gourd, bitter gourd and pumpkin of Vellanikkara and those from snake gourd, ridge gourd, bottle gourd and bitter gourd of Elanad produced conspicuous concentric zonations in pure culture. Similar type of colony characters of *Alternaria* have been reported earlier by Venkatakrishniah (1953) and Bhargava and Singh (1985).

Variation in the growth rate of thirteen isolates of *A. alternata* and *A. tenuissima* were tested in five different media. The isolates of *Alternaria* recorded good growth on PDA medium and took 8-12 days to complete full growth. All isolates of *A. alternata* recorded the same growth rate on PDA and host leaf extract agar medium except the isolate from ash gourd of Elanad on PDA, which took the minimum time of eight days to complete the full growth. *A. alternata* isolate of bottle gourd and pumpkin of Vellanikkara location took 16 days on Richard's agar medium to complete full growth. So variation in growth on different media tested was exhibited by various isolates of *A. alternata*. Similarly *A. tenuissima* isolates of bitter gourd from both the location showed much variation in growth on different media.

All isolates of *Alternaria* sporulated well on PDA medium and spore count ranged from 2.5 to 4.9 x 10^5 spores ml^{-1}. *A. alternata* isolate of ridge gourd from Vellanikkara produced maximum spores and minimum spores were produced by *A. alternata* isolate of ivy gourd from Elanad. Singh *et al.* (2001a) studied the effect of different media on growth and sporulation of *A. alternata* causing *Alternaria* blight of chickpea and found maximum colony size on potato dextrose agar medium followed by Potato mashed dextrose agar. Davis (2003) also reported PDA as best media for the growth of *A. alternata* from ivy gourd.

Morphological characters of different isolates of *Alternaria* were studied and all isolates were found to produce conidiophores and conidia in pure culture. In general mycelium are branched, light brownish grey to dark brown measuring 4.03-4.06 µm in breadth with septate at 16.12-32.45 µm intervals. Conidiophores branched, seen in single or groups with 48.72-113.68 µm length and 3-9 septa. All isolates produced conidia in chain, conidia straight, obclavate, measuring 12.09-89.32 x 4.03-16.24 µm with 3-8 transverse and 1-5 longitudinal septa, beak prominent measuring 4.03-48.72 µm with 1-3 septa. The conidia of isolates of bitter gourd of both locations and that of snake gourd of Elanad and ivy gourd of Vellanikkara were smooth. Occasionally branching of conidial chain was observed. Few transverse septa of conidia of these isolates were thicker than their longitudinal septa. The conidia of isolates from ash gourd, ridge gourd, bottle gourd and pumpkin of both locations and snake gourd of Vellanikkara and ivy gourd of Elanad area were either smooth or prominently verruculose and produced in straight chain. No difference in the thickness of transverse and longitudinal septa was observed. Variations in the size of conidial chain were also noticed. Out of the 14 isolates 10 of them produced conidia in short chain and remaining four in long chains. These mycelial, conidial and conidiophore characters were comparable to that described by Ellis (1971) and Subramanian (1971) for *A. alternata* and *A. tenuissima*. So based on these characters coupled with their pathogenicity in the respective hosts, isolates of *Alternaria* from different cucurbitaceous vegetables from both the locations were found to be caused by two species of *Alternaria* and they were identified as, *A. alternata* (Fr.) keissler and *A. tenuissima* (kunze ex pers.) Wiltshire. *A. alternata* was found to cause leaf blight in ash gourd, ridge gourd and bottle gourd of Vellanikkara and Elanad area. It was also found to be pathogen of snake gourd of Vellanikkara and ivy gourd of Elanad area respectively. The leaf blight disease of bitter gourd of both the locations, snake gourd of Elanad area and ivy gourd of Vellanikkara was caused by *A. tenuissima*. Earlier workers reported the same two species of *Alternaria* as leaf blight pathogen on different cucurbitaceous vegetables. *A. alternata* was reported on watermelon by Narain *et al.* (1985), on cucumber by Vakalounakis and Malathrakis (1988), on watermelon and cucumber by Urbanzki *et al.* (2003), and on ivy gourd by Davis (2003). *A. tenuissima* was reported by Kumar *et al.* (2001) on *Coccinia grandis* as leaf spot pathogen.

Perusal of literature revealed no reports of *Alternaria* infection on ash gourd, snake gourd and pumpkin from India. In bottle gourd *Alternaria cucumerina* was reported as pathogen of leaf blight disease. *A. tenuissima* and *A. alternata* has been reported as the causal organism of leaf blight disease of bitter gourd and ivy gourd. Hence it is the first report of *A. alternata* (Fr.) Keissler on ash gourd, snake gourd, bottle gourd and pumpkin and *A. tenuissima* (Kunze ex Pers.) Wiltshire on snake gourd as the pathogens of leaf blight disease from India.

The morphological characters of isolates of *A. alternata* and *A. tenuissima* were subjected to cluster analysis. Clustering was done as per UPGMA method of Sneath and Sokal (1973) and the clustering pattern of isolates revealed a degree of variability. The 14 isolates were grouped into four clusters, A, B, C and D. The cluster A consisted of 3 subclusters, the lowest dissimilarity index was noticed in sub cluster A_2 between *A. alternata* isolates of ash gourd and snake gourd of Vellanikkara location. *A. tenuissima* of bitter gourd and ivy gourd from Vellanikkara location were found more similar in their morphological characters and were grouped under the sub cluster A_1. *A. tenuissima* of snake gourd and bitter gourd of Elanad area stands separate and the latter showed maximum dissimilarity with other isolates.

An investigation was carried out to find out the effect of various physiological and nutritional factors such as temperature, p^H, light, carbon and nitrogen sources on growth of isolates of *Alternaria* from cucurbitaceous vegetables. It was found that the temperature had an influence on the growth of *Alternaria* isolates. Among the temperatures tested maximum fresh weight and dry weight of the isolates from Vellanikkara was at 25°C, while it was 30°C for Elanad isolates. However temperature had no significant effects on the dry weight of isolates. *A. alternata* isolates from Elanad recorded more fresh weight than that from Vellanikkara, and among them *A. alternata* from ridge gourd recorded the maximum fresh weight at 30°C. All isolates of *A. alternata* from both locations except from ash gourd of Vellanikkara recorded higher fresh weight value at 25° and 30° than at 35°C. The same trend was noticed in the case of

A. tenuissima isolates except in bitter gourd from Elanad which recorded minimum fresh weight at 25°C. So it can be concluded that isolates of *A. alternata* and *A. tenuissima* preferred a temperature range of 25-30°C which is supportive to the earlier reports (Saeed *et al.*, 1995; Kumari *et al.*, 1998; Maheshwari *et al.*, 2000a; Singh *et al.*, 2001b).

Most of the fungi prefer acidic p^H because they are more adaptable to hydrogen ion activity than bacteria (Singh, 2003). But they can tolerate the p^H range in which their hosts normally grow. Hence, a study was done to find out the effect of p^H on the growth of isolates of *Alternaria*. Slightly acidic to neutral p^H was found favourable for the maximum growth of fungal isolates. The isolates recorded maximum fresh weight at p^H 6 which was on par with that at p^H 7. Maximum dry weight was at p^H 6 for the isolates from Vellanikkara while it was at p^H 7 for Elanad isolates. However individual isolates from both places showed same preference to a particular p^H. So it can be concluded that the isolates of *A. alternata* and *A. tenuissima* prefer a p^H range of 6 to 7 for maximum mycelial growth in pure culture. Earlier studies also were in line with present one (Ayub *et al.*, 1995; Hossain *et al.*, 1997; Jash *et al.*, 2003).

Light influence the development of plant diseases as it affect the survival of infective propagules of the pathogen, incubation period, secondary inoculum production and sometimes symptom expression. In this context, the influence of light on the growth of isolates of *Alternaria* was evaluated and it was noticed that the isolates from Vellanikkara and Elanad recorded maximum fresh weight of mycelial growth under full light and full dark respectively, but was on par with fresh weight recorded under alternate light and dark. Observation on dry weight of mycelium of isolates from Vellanikkara also showed the same trend; however the maximum dry weight of Elanad isolates was under full light. Variation was observed in the fresh weight of different isolates of both *A. alternata* and *A. tenuissima* from Elanad, which recorded more fresh weight in all three treatments compared to the fresh weight recorded by isolates of Vellanikkara. The same trend was observed in dry weight of mycelial growth recorded by the isolates of both the locations. It was also observed that variation in the fresh weight of mycelial growth due to the effect of light was observed among the isolates of *A. alternata* and

A. tenuissima. It was concluded that *A. alternata* and *A. tenuissima* prefer either full light or alternate light and dark for maximum growth in culture which is in agreement with that of earlier reports. Choulwar and Datar (1991) reported that the exposure of *A. solani* causing leaf blight of tomato to light produced maximum growth and distinct zonations, but no sporulation. In, 1995 Ayub *et al.* found out that the 12 h cycles of light and darkness supported the maximum growth of *A. cyamopsidis* causing leaf blight of guar. Chen *et al.* (2000) also found out that the conidial germination of *A. tenuis* was maximum at 12 h light and 12 h darkness.

Among the different carbon sources tested for the best growth of different isolates of *Alternaria* the medium incorporated with starch recorded maximum fresh and dry weight of isolates of Vellanikkara. The isolates from Elanad recorded maximum fresh weight on starch containing medium, but maximum dry weight was recorded by the medium containing lactose. All isolates of *A. alternata* and *A. tenuissima* except *A. tenuissima* of ivy gourd recorded maximum fresh weight in starch containing medium. So no variation except one isolate of *A. tenuissima* was observed among the isolates of *Alternaria* and hence starch is selected as the best carbon source for maximum growth of *Alternaria* sp. Jash *et al.* (2003) reported starch as best source of carbon for supporting good growth of *A. zinniae*. But Thind (1997) reported lactose as best carbon source for the growth of *A. alternata*.

With respect to nitrogen sources, KNO_3 was found to be the best for supporting the maximum fresh weight of isolates from Vellanikkara, which was on par with $MgNO_3$. The maximum dry weight was recorded in media containing $MgNO_3$ which was on par with KNO_3. In the case of isolates from Elanad, no significant difference between nitrogen sources in fresh weight was noticed, but $NaNO_3$ containing medium recorded maximum dry weight. All isolates of *Alternaria* from Elanad area except the isolate from snake gourd recorded more fresh weight compared to the isolates from Vellanikkara. Same trend was observed in dry weight also. Among the nine isolates of *A. alternata*, six isolates recorded maximum fresh weight on medium containing KNO_3. Whereas in case of *A. tenuissima* two isolates, one from bitter gourd and another from snake gourd of Elanad area recorded maximum fresh weight in KNO_3 media and the other two isolates performed well on medium containing $MgNO_3$ as nitrogen source. So variation among the isolates of *A. alternata* and *A. tenuissima* was noticed in their preference to nitrogen source for maximum growth in culture. *A. alternata* isolate from ridge gourd only recorded maximum fresh weight in medium with $NaNO_3$, but it was on par with fresh weight in KNO_3 incorporated media. From all these observations, it was found that *Alternaria* spp. could utilize either KNO_3 or $MgNO_3$ as nitrogen source for the best growth in medium. Earlier many workers reported KNO_3 as best nitrogen source for supporting good growth of different species of *Alternaria* (Ayub *et al.*, 1995; Saeed *et al.*, 1995 and Khatun *et al.*, 1996; Hossain *et al.*, 1997).

Thus from the results of cultural, morphological, physiological and nutritional characters, it was found that *A. alternata* and *A. tenuissima* were the main pathogens causing leaf blight disease in cucurbits. Isolates of *A. alternata* and *A. tenuissima* recorded good growth rate on PDA and host leaf extract agar media. Different isolates produced brownish grey to dark brown aerial mycelium in pure culture with conspicuous concentric zonations except in isolates from snake gourd, bottle gourd and ivy gourd of Vellanikkara and ash gourd, ivy gourd and pumpkin of Elanad. Cluster analysis of different morphological characters of various isolates of *A. alternata* and *A. tenuissima* revealed a degree of variability among these isolates. The isolates of *A. alternata* and *A. tenuissima* from both locations recorded good growth in temperature range of 25-30°C, required a slight acidic to neutral p^H, alternate light and dark and full light for good growth. All these isolates showed good growth in medium with starch as carbon source and could utilize KNO_3 or $MgNO_3$ as nitrogen source.

A study on conidial germination of different isolates of *Alternaria* isolated from different cucurbitaceous vegetable was conducted. Conidial germination is an important factor and is considered as the pre infection stage in the pathogenesis of fungi. In fungi, the conidia or spores when come in contact with the host, germinate in the presence of moisture and produce germ tube or infection peg. In certain fungi, their spores geminate and produce appressoria and on which infection pegs are formed. In this study all isolates of *Alternaria* initiated the germ tube formation at three hours after incubation and cent per cent germination was recorded at 10 h after incubation. All the cells of conidium germinated by producing germ tube which elongated to form hypha. But the appressorium production was not observed in these isolates. Earlier many workers studied on the rate of spore germination of *Alternaria* spp. (Benlioglu and Delen, 1996; Chen *et al.*, 2000 and Raranciuc, 2002). A similar study was conducted by Davis (2003) and she found that the spores of *A. alternata* obtained from ivy gourd germinated three hours after incubation and noticed germination of all cells of conidium by producing germ tube which elongated to form hypha.

Compatibility between different isolates of *Alternaria* was studied. Hyphal growth of all the isolates were found mingling when they grown together on the medium. The result showed the compatible nature of different isolates of *A. alternata* and *A. tenuissima* and any type of antagonistic activity was not observed between the isolates. All the isolates produced spores profusely on the cellophane paper. On search of literature no information on the compatibility study between different species of *Alternaria* was obtained.

Histopathological changes brought about by *Alternaria* spp. on different cucurbitaceous vegetables were studied. *Alternaria* infection caused severe distortion of epidermal cells and mesophyll layer. Conidiophores were found emerging from the damaged epidermis. Infection affected the distribution pattern of chloroplast, disintegration of chlorophyll and caused complete death of cells. No variation was observed on the histopathological changes occurred due to infection by different isolates of *A. alternata* and *A. tenuissima* in their respective hosts. Similar type of studies was conducted by many workers. Munjal and Gupta (1965) observed during the study of host parasite relations of the anthracnose of celosia that the cells of diseased tissue lost their shape and later the hyphae collected underneath the epidermis form stroma, from which conidiophore were produced. Hyphal structures of *A. zinniae* were observed in the cross sections of inflorescences of marigold (*Calendula officinalis*) by Karlatti and Hiremath (1989). Histology and development of *A. helianthi* in sunflower leaves were studied by

Thaiz and Subero (1997) and histopathological changes due to *A. alternata* infection on ivy gourd was studied by Davis (2003).

The next part of the investigation was to found out the host range of various isolates of *Alternaria* because host range plays an important role in the life cycle of a fungus for its survival and spread. To find out the host range of *Alternaria* isolated from their respective host, cross inoculation was done on different cucurbitaceous vegetables *viz.*, ash gourd, snake gourd, ridge gourd, bottle gourd, bitter gourd and pumpkin, solanaceous vegetables *viz.*, tomato, chilli, brinjal and other vegetables *viz.*, amaranthus, cowpea and bhindi. All cucurbitaceous, solanaceous and other vegetables were found susceptible to all isolates of *Alternaria* spp. and produced typical leaf blight symptom on all the crops inoculated. Symptom appeared as yellow discolouration on the inoculated position. Later it changed to brown and enlarged in size with characteristic yellow halo. In this case of all isolates of *Alternaria*, the symptom first developed on the inoculated leaves given pinpricks on lower surface. So an injury is required for easy entry of the pathogen into the host and took less incubation period for symptom expression. From the result it was observed that there was no difference in the incubation period of different isolates of *Alternaria* in cucurbitaceous, solanaceous and other vegetables tested. These observations indicate that the various isolates of *A. alternata* and *A. tenuissima* were pathogenic to all cucurbitaceous, solanaceous and other vegetables tested and these vegetables could act as collateral hosts and could help in the perpetuation and spread of *Alternaria* spp. Vakalounakis in 1990b conducted host range study of *A. alternata* f. sp. *cucurbitae* and reported that 27 species of cucurbitaceae were susceptible to *A. alternata* f. sp. *cucurbitae* causing leaf spot of cucumber.

Toxins produced by the plant pathogenic organisms also have an important role in the development of disease. A micro organism cannot be pathogenic unless it is toxigenic. This means unless the organisms produce toxins, it cannot cause disease even when it penetrate the host and damage the cells.

In this study exotoxin and endotoxin were extracted from the various isolates of *Alternaria* from two locations, grown on broth culture and spot inoculated on their respective host as well as cross inoculated on cucurbitaceous vegetables, solanaceous and other vegetables *viz.*, amaranthus, cowpea and bhindi. The results revealed a positive reaction by all crops inoculated with toxic metabolites of various isolates of *Alternaria*. The initiation of symptom was first noticed on leaves inoculated with exotoxin of *Alternaria* and it took very short incubation period of two days for the symptom expression. Not much variation in the symptom development by the exotoxin of various isolates of *A. alternata* and *A. tenuissima* was noticed. In bhindi as in the case of host range study, small irregular blighted areas were developed on the inoculated leaves, which later enlarged and covered most of the leaf area and complete blightening of leaves observed.

The leaves inoculated with endotoxin of isolates of *A. alternata* and *A. tenuissima* produced characteristic yellowing on the leaf margin and later complete blightening of leaves occurred. It took more incubation period of four days for the symptom expression. No variation in the development of symptom by endotoxin of various isolates of *A. alternata* and *A. tenuissima* was noticed.

From these observations, it was clear that various isolates of *A. alternata* and *A. tenuissima* isolated from cucurbitaceous vegetables of two locations have the ability to initiate pathogenesis by producing toxins. Moreover, exotoxin and endotoxin of these isolates did not show any host specificity and they were even capable of causing leaf blight on vegetables other than cucurbits. Earlier report (Singh, 2003) indicated the production of non host specific toxin by *A. alternata* and development of striking variegated chlorosis in cucumber, citrus and other plants by both toxin and pathogen. The effectiveness of exotoxin of *A. alternata* in pathogenesis of leaf blight of ivy gourd reported by Davis (2003) also supported this result.

Summing up the discussion so far it may be concluded that *A. alternata* and *A. tenuissima* were identified as the important species of *Alternaria* causing leaf blight disease in cucurbits. Variability in symptom expression was observed among the isolates of *A. alternata* and *A. tenuissima* in producing concentric zonations. Not much variation was observed in the colour and thickness of the colony growth of different isolates of *Alternaria* whereas variability was seen in the formation of concentric zonations in the culture. Different isolates of *A. alternata* and *A. tenuissima* showed variation in growth rate in different media tested. Variability was observed in the conidial characters of *A. alternata* and *A. tenuissima*. The microscopic measurements of various structures of different isolates of *A. alternata* and *A. tenuissima* were comparable to the description given in authentic books.

Cluster analysis of different morphological character of *Alternaria* isolates revealed a degree of variability among the isolates. *A. tenuissima* isolates of snake gourd and bitter gourd of Elanad area stands separate and showed more dissimilarity with all other isolates. Whereas *A. tenuissima* isolates of bitter gourd and ivy gourd of Vellanikkara location showed more similarity with the *A. alternata* isolates of ash gourd from Elanad and ridge gourd of Vellanikkara location.

In general no variability among isolates of *A. alternata* and *A. tenuissima* was observed in their physiological characters. The host range study of both the isolates of *A. alternata* and *A. tenuissima* showed that they were pathogenic to all cucurbitaceous, solanaceous and other vegetables tested. Hence no variability with regard to the host range was recorded among the isolates of *A. alternata* and *A. tenuissima*. Effect of exotoxin and endotoxin of various isolates of *Alternaria* on pathogenesis also showed no variation among the isolates of *A. alternata* and *A. tenuissima*.

The present investigation on the variability of *Alternaria* isolates causing leaf blight of cucurbitaceous vegetables have enriched our knowledge in various aspects especially etiology of leaf blight diseases of cucurbits, symptomatology, morphological and cultural characters of the pathogens, their physiological and nutritional characters, host range and efficiency of toxin in pathogenesis.

Summary

6. SUMMARY

The present investigation on "Variability of *Alternaria* isolates causing leaf blight diseases in cucurbits" was carried out to study various aspects particularly symptomatology, cultural, morphological, physiological and nutritional characters, host range and effect of toxin on disease development and to understand the variability existing among the isolates of *Alternaria*.

A survey was conducted at two different locations *viz*., vegetable field of Olericulture Department, Vellanikkara and farmer's field at Elanad, Thrissur district. Isolation of diseased specimens yielded fungal growth on PDA and isolated seven cultures from Vellanikkara and six from Elanad area. The Pathogenicity of these isolates was proved by artificial inoculation under *in vitro* and *in vivo* conditions.

Studies on symptomatology of various leaf blights collected from the two locations *viz*., Vellanikkara and Elanad showed similarities in symptoms produced by the respective isolates. *Alternaria* infection was noticed on older leaves. The symptom or infection usually started from leaf margins as small, irregular brown lesions. Later brown lesions enlarged in size with characteristic yellow halo. Characteristic concentric zonations were noticed on symptoms produced on ash gourd, bitter gourd and ivy gourd. Concentric zonations were absent in the symptom produced on snake gourd, ridge gourd, bottle gourd and pumpkin. So only variation in symptom expression was with respect to the presence or absence of concentric zonations

To find out the variation existing among isolates of *Alternaria,* cultural and morphological characters of all the isolates were studied in detail on PDA medium. The different isolates of *Alternaria* produced thick and velvety colony with brownish grey to dark brown aerial mycelium. The isolates showed variation in colony character only with respect to the presence of concentric zonations in the culture. Isolates from ash gourd, ridge gourd, bitter gourd and pumpkin of Vellanikkara location and from snake gourd, ridge gourd, bottle gourd and bitter gourd of Elanad location produced concentric zonations in the medium.

All the isolates sporulated well on PDA medium and spore count ranged from 2.5 to 4.9 x 10^5 spores ml^{-1}. Ridge gourd isolate from Vellanikkara produced maximum spores and minimum spores were produced by the isolate of ivy gourd from Elanad area.

Variation in growth rate of different isolates of *Alternaria* was studied in five different media. The results revealed that PDA supported maximum growth of all isolates. They took 8-12 days to complete 9 cm growth in Potato dextrose agar medium. On all media bitter gourd isolates took maximum time for attaining the full growth. Isolates of *Alternaria* of the two locations showed maximum variation in growth on Richard's agar medium.

Study on morphological characters of *Alternaria* spp. revealed that all the isolates were found to produce conidiophores and conidia in pure culture. Mycelium are branched, brown measuring 4.03-4.06 µm in breadth with septate at 16.12-32.45µm intervals, conidiophores branched seen in single or group with 48.72-113.68µm length and 3-9 septa, conidia are straight, obclavate, 12.09-89.32 x 4.03-16.24 µm with 3-8 transverse and 1-5 longitudinal septa and beak measuring 4.03-48.72µm with 1-3 septa. The conidia of bitter gourd isolates of both locations, snake gourd of Elanad and ivy gourd of Vellanikkara were smooth. The transverse septa of the conidium of bitter gourd isolates were thicker than their longitudinal septa. Variations in the size and branching of conidial chain were also noticed. Based on cultural and morphological characters, the isolates were identified as *Alternaria alternata* (Fr.) Keissler and *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire. Search of literature revealed no reports of these species of *Alternaria* on ash gourd, snake gourd, bottle gourd and pumpkin and hence it is the first report of *Alternaria alternata* (Fr.) Keissler on ash gourd, snake gourd, bottle gourd and pumpkin and also *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire on snake gourd as the causal organisms of leaf blight disease from India.

Cluster analysis of morphological characteristics revealed a degree of variability among the isolates. The lowest dissimilarity index was noticed in sub cluster A_2 between *A. alternata* isolates of ash gourd and snake gourd of Vellanikkara location. *A. tenuissima* of bitter gourd and ivy gourd from Vellanikkara were found more similar in their morphological characters. *A. tenuissima* of snake gourd and bitter gourd of Elanad area stands separate and the latter showed maximum dissimilarity with other isolates.

Study on the effect of physiological and nutritional characters such as temperature, p^H, light, carbon and nitrogen sources on fresh and dry weight of different isolates of *Alternaria* revealed that, among the different temperatures tested 25 to 30°C was found to be the most favourable temperature range for the growth of different *Alternaria* isolates and the p^H levels 6 and 7 supported the growth of most of the isolates. Among the light treatments full light and alternate dark and light were found to be better for the growth. Medium containing starch as the carbon source and KNO_3 and $MgNO_3$ as the nitrogen source supported maximum growth of the various isolates of *Alternaria.*

Study on conidial germination showed that, all the isolates of *Alternaria* started germination at three hours after incubation and cent per cent germination was observed at 10 h after incubation. During germination germ tubes were produced from all the cells of conidia which developed into hyphae.

In vitro compatibility study between different isolates of *Alternaria* revealed that, all the isolates were compatible with each other when they were grown together over cellophane paper in a Petri dish containing plain agar medium. All the isolates grown well and produced spores profusely on the cellophane paper.

Histopathological changes occurred due to *Alternaria* infection was similar in all the cucurbitaceous vegetables tested. The infection leads to the destruction of epidermal and mesophyll cells. Infection affected the distribution pattern of chloroplast and caused disintegration of chlorophyll grains. Conidiophores and hyphae were found protruded through the broken epidermis. Complete necrosis and death of whole cells were noticed during advanced stage.

Host range study was conducted by cross inoculating different isolates of *Alternaria* on to various cucurbits, solanaceous crops and other vegetables *viz.*, amaranthus, cowpea and bhindi. All the isolates produced symptom on all the crops tested. Symptom appeared as yellow discolouration on the inoculated position. Later yellow discolouration changed to dark brown in colour enlarged in size with characteristic yellow halo. Whereas in bhindi, small irregular blighted areas developed on leaves which enlarge in size gradually and cover most of the leaf area with in three to five days after the symptom appearance.

The exotoxin and endotoxin extracted from the culture of different *Alternaria* isolates was spot inoculated separately on leaves of different cucurbitaceous, solanaceous and other vegetables to study the effect of toxin on disease development. Exotoxin inoculated leaves showed the symptom first as yellowing on the inoculated position followed by brown lesions after five days of inoculation. Gradually brown lesions cover most of the leaf area. Leaves inoculated with endotoxin produced symptom six days after incubation. Yellowing was noticed below the inoculated position and blightening started from the margin which later enlarged in size and spread to whole leaf.

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* Originals not seen

Appendix

APPENDIX -1

MEDIA COMPOSITION

1. POTATO DEXTROSE AGAR MEDIUM

Potato	: 200 g
Dextrose	: 20.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml

2. HOST LEAF EXTRACT AGAR MEDIUM

Host leaf	: 300 g
Agar	: 20.0 g
Distilled water	: 1000 ml

3. SELECTIVE MEDIUM FOR *ALTERNARIA*

Sucrose	: 20.0 g
$CaCO_3$	: 30.0 g
Agar	: 20.0 g

4. RICHARD'S AGAR MEDIUM

Sucrose	: 50.0 g
KNO_3	: 10.0 g
KH_2PO_4	: 5.0 g
$MgSO_4.7H_2O$	: 2.5 g
$FeCl_3 .6 H_2O$	: 0.02 g
Agar	: 20.0 g
Distilled water	: 1000 ml

5. CZAPEK (DOX) AGAR MEDIUM

Sucrose	: 30.0 g
$NaNO_3$	: 2.0 g
$FeSO_4.7H_2O$	: 0.01 g
K_2HPO_4	: 1.0 g
$MgSO_4.7H_2O$	: 0.5 g
KCl	: 0.5 g
Agar	: 20.0 g
Distilled water	: 1000 ml

VARIABILITY OF *Alternaria* ISOLATES CAUSING LEAF BLIGHT DISEASES IN CUCURBITS

By

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ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

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ABSTRACT

Alternaria leaf blight is one of the serious diseases of cucurbits in Kerala. A study on "Variability of *Alternaria* isolates causing leaf blight diseases in cucurbits" were conducted at College of Horticulture, Vellanikkara during 2004 - 2005 and they include symptomatology, cultural, morphological, physiological and nutritional characters, host range and effect of toxin on disease development and also on the variability if any existing among the different isolates of *Alternaria*.

Isolation of *Alternaria* from diseased samples of ash gourd, snake gourd, ridge gourd, bottle gourd, bitter gourd, ivy gourd and pumpkin collected from Vellanikkara and Elanad areas of Thrissur district was done in Potato dextrose agar medium and isolation of diseased specimens yielded fungal growth similar to *Alternaria* sp. and obtained seven isolates from Vellanikkara and six isolates from Elanad. Symptom or infection usually started from leaf margins as small, irregular brown lesions. Later brown lesions enlarged in size with characteristic yellow halo. Variation in symptom expression was noticed only with respect to the presence or absence of concentric zonations. Ash gourd, bitter gourd and ivy gourd produced symptoms with concentric zonations on the infected area.

Variation in cultural and morphological characters of different isolates of *Alternaria* was studied. *Alternaria* spp. produced thick and velvety colony with brownish grey to dark brown aerial mycelium and the isolates showed variation in colony character only with respect to the presence of concentric zonations in the culture. All isolates sporulated well on Potato dextrose agar medium and spore count ranged from 2.5 to 4.9 x 10^5 spores ml^{-1}. Ridge gourd isolate from Vellanikkara produced maximum spores and minimum spores were produced by the isolate of ivy gourd from Elanad. Variation in growth rate was observed among different isolates of *Alternaria* grown on different media. Among the different media tested Potato dextrose agar supported maximum growth of different isolates. All the isolates were found to produce conidiophores and conidia in pure culture. Variations in the size and branching of conidial chain were also noticed and size of conidial chain varies from 2-12.

Cultural and morphological characters revealed that *Alternaria alternata* (Fr.) Keissler and *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire was the two main species of *Alternaria* causing leaf blight diseases in various cucurbitaceous vegetables. There was no earlier reports of these species of *Alternaria* on ash gourd, snake gourd, bottle gourd and pumpkin and hence it is the first report of *Alternaria alternata* (Fr.) Keissler on ash gourd, snake gourd, bottle gourd and pumpkin and also *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire on snake gourd as the pathogen of leaf blight diseases from India.

Cluster analysis of morphological characteristics revealed a degree of variability among the isolates. The lowest dissimilarity index was noticed between *A. alternata* isolates of ash gourd and snake gourd of Vellanikkara. *A. tenuissima* of bitter gourd and ivy gourd from Vellanikkara found more similar in their morphological characters. *A. tenuissima* of snake gourd and bitter gourd of Elanad area stands separate and the latter showed maximum dissimilarity with other isolates.

An *in vitro* study on the effect of physiological and nutritional characters such as temperature, p^H, light, carbon and nitrogen sources on different isolates of *Alternaria* were carried out and observed that, among the different temperatures tested, 25 to 30°C was found to be the most favourable temperature range for the growth of different *Alternaria* isolates and the p^H levels 6 and 7 supported the growth of most of the isolates. Among the light treatments full light and alternate light and dark were found to be better for the growth, medium containing starch as the carbon source and KNO_3 and $MgNO_3$ as nitrogen source supported maximum growth of different isolates of *Alternaria.*

Study on conidial germination revealed that all isolates took three hours for starting germination and 10 h for cent per cent germination. All cells of conidium germinated by producing germ tube which elongated to form hyphae.

All isolates of *Alternaria* species obtained from different cucurbitaceous vegetables from both the locations were found to be compatible with each other when grown together.

Histopathological changes brought about by all the isolates of *Alternaria* were almost similar. Due to infection epidermal cells get destructed and hyphae and conidiophores of the fungus seen emerging through the broken epidermis. Destruction of chloroplast and necrosis and death of complete cells occurred in the final stages.

Host range study proved that all the isolates of *Alternaria* from both the locations produced symptoms on all the cross inoculated cucurbits. They were also found producing symptoms on solanaceous vegetables and other vegetables *viz*., amaranthus, cowpea and bhindi.

From the toxin study, it was found that both the exotoxin and endotoxin were effective in producing symptoms on all cucurbitaceous, solanaceous and other vegetables tested. The exotoxin produced the symptoms first.