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MORPHOLOGY AND STATUS OF OCCURRENCE OF ANTHRACNOSE OF BEAN (*PHASEOLUS VULGARIS* L.) CAUSED BY *COLLETOTRICHUM LINDEMUTHIANUM* (SACC. AND MAGN.) SCRIB. IN KASHMIR VALLEY

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ABSTRACT
Survey of important bean growing areas of Kashmir Valley was carried out to assess the disease incidence and intensity of anthracnose of beans. The disease was prevalent in all the potential bean growing pockets of the valley surveyed during the course of survey. The incidence ranged from 23.51% to 82.14% with disease intensity ranging from 8.75 to 33.38%. Highest disease incidence (34.81% and 51.11%) and intensity (13.73 and 21.91%) on leaves and pods was recorded in district Anantnag, with least in district Shopian (24.80% and 28.99%; 9.66% and 11.93%). The pathogen associated with the disease was isolated and identified as *Colletotrichum lindemuthianum*(Sacc and Magn.) Scrib. Morphological studies of the fungus revealed that conidia are hyaline, single celled dumbbell shaped born in acervuli bearing setae. The dimensions of the conidia on culture and host varied from 9.5-11.5 x 3.5-4.5(10.5x 4) and 12-22.0 x 4-6 (17.25 x 4.25), respectively. The fungus produces acervuli with its dimensions ranging from 140-320 (204.14) and 175- 285 (270) on culture and host respectively. It was also observed that the acervulibearing characteristic appendages on its surfaces that measure 64-108 x 2-4 (84.22 x 3) and 60- 120 x 5-6 (90 x 5.55)on culture and host respectively.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) belongs to family *Leguminaceae* and occupies a premier place among grain legumes in the world including India, where it is locally called as *Rajmash* (Sharma *et al*., 1994). The crop is distributed worldwide and can grow under diverse agro-ecosystems ranging from tropical, sub-tropical to temperate region (Popelka *et al*., 2004). In Jammu and Kashmir it is cultivated over an area of 2000 ha with an annual production of 400 tons (Masoodi and Masoodi, 2003).

Because of the diverse production conditions and vast area grown, beans suffer from a number of disease, some of them taking heavy toll on the crop and limit the production and productivity. Among this anthracnose of beans warrants a serious attention as it inflicts heavy economic losses to the crop, which may reach up to 100%, under conducive conditions (Silva *et al*., 2007; Sharma *et al*., 2008). In India disease incidence has been reported to vary between 24.59 to 51.72 per cent (Sharma and Sugha, 1995). Qandah and Al-Momany (2003) demonstrated disease incidence ranging from 0 to 50 per cent in different parts of Jordan valley. In Jammu region of Jammu and Kashmir state disease incidence of 63.46 was recorded by Kalha *et al*. (2005). The disease was reported for the first time in valley by Khan *et al*. (2009) as a disease of minor importance but during the last few years bean anthracnose has appeared as a potential threat to the bean production, especially in high alititudes were presence moisture throught the crop season provides a conducive environment for pathogen growth and multiplication. No systematic work has been done in the valley on this disease, hence the present studies were carried out with the aim to establish the status of the disease in the valley and to study the morphology of the causal pathogen.

MATERIALS AND METHODS

Status of disease

For recording incidence and intensity of anthracnose of beans regular survey of potential bean growing pockets from Anantnag, Bandipora, Ganderbal and Shopian districts of Kashmir province was conducted during *kharif* cropping season. The areas where beans have been intensively and extensively cultivated for years were selected in each district. The survey was conducted at seedling and pod maturity stage of the crop. The variety under survey was the most commonly grown variety locally known as Tripashi Rajmah.

Assessment of disease incidence

Each district was divided into three villages and in each village three bean fields were selected randomly. The disease incidence was recorded by randomly selecting three sites of 2

x 1 m size from each selected field. At each selected site, 10 plants on an average were taken into consideration. The per cent disease incidence of anthracnose of beans at each survey plot was duly recorded on leaves and pods and accordingly computed and calculated by employing the following formula:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected leaves/pods}}{\text{Total number of leaves/pods examined}} \text{ X } 100$$

Assessment of disease intensity

Percent disease intensity was estimated by using the 0-9 scale proposed by Mayee and Dattar (1986) (Plate 1) with slight modifications, and was calculated by the formula:

$$\text{Percent disease intensity} = \frac{\sum (n \text{ xv})}{N \text{ x } G} \text{ X } 100$$

Where:

$\sum$ = Summation

n = Number of diseased leaves/pods in each category

v = Category value

N = Total number of leaves/pods examined

G = Highest category value

Score	Description
0	No symptoms on leaf/pods
1	Small, round brown spots covering 1% or less of leaf/ pod area
3	Brown, sunken spots covering 1- 10 % of leaf/pod area
5	Brown spots enlarging to form circular spots covering 11-25% of leaf/pod area
7	Circular brown, sunken spots, covering 26 – 50% of leaf/ pod area
9	Circular to irregular, brown sunken spots covering 50% or more of the leaf/pod area

Laboratory studies and characterisation of the causal pathogen of Anthracnose of beans

Isolation of pathogen

Isolation of the pathogen was performed from the leaves and pods of bean plants collected from the surveyed locations and showing typical disease symptoms. Isolation of pathogen was done from infected plant parts of leaves and/or pods along with some margin of healthy tissues around it. The bits were then washed with water and simultaneously surface sterilized by immersing in freshly prepared 0.1% aqueous mercuric chloride solution for 30 seconds, followed by 3-4 times washings with sterile distilled water. These were aseptically transferred onto sterilized petri plates containing potato dextrose agar (PDA) medium and incubated at 24 ± 1ºC for 7 days. The plates were regularly monitored and periodically observed for fungal growth and subsequently sub-cultured on PDA medium. Pure culture was obtained using single spore technique. Pathogenicity was proved following the Kochs postulates.

Morphology of fungus

The morphological characteristics of fungus were studied in laboratory on host as well as on artificial culture. Semi-permanent slides were prepared from seven days old culture stained with cotton blue in lactophenol. The slides were examined under microscope (40 xs) with respect to following characters:

Colony: Colour, shape and mycellial growth

Conidia: Size, shape, colour, septation

Setae: Size, shape, colour, septation

Acervuli: Size, shape, colour

RESULTS

Occurrence and severity of disease

Table 1: Incidence of anthracnose of beans at various locations in Kashmir valley during 2011

District	Location	Per cent disease incidence*	
		Leaves	Pods
Anantnag	Larnoo	53.79 (47.18) **	82.14 (65.01)
	Danter	24.64 (29.72)	32.70 (34.87
	Momin	26.02 (30.04)	39.35 (38.82)
	Mean	34.81 (35.64)	51.11 (46.23)
Bandipora	Chatibandi	28.15 (32.04)	43.51 (41.27)
	Bandipora	24.65 (29.75)	29.74 (33.03)
	Sumbal	23.51 (29.00)	25.65 (30.42)
	Mean	25.43 (30.26)	32.96 (34.90)
Ganderbal	Batwani	27.05 (31.31)	43.38 (41.19)
	Yangura	29.64 (32.98)	57.79 (49.48)
	Zazun	27.02 (31.30)	40.98 (39.80))
	Mean	27.90 (31.86)	47.38 (43.98)
Shopian	Dradkralipora	24.48 (29.65)	28.19 (32.07)
	Herman	24.11 (29.40)	26.90 (31.23)
	Sedav	25.83 (30.54)	31.88 (34.37)
	Mean	24.80 (29.86)	28.99 (32.56)
Overall mean	28.47 (32.10)	40.18 (39.29)	
$CD_{(Pd^* 0.05)}$	District	1.59	1.73
	Location	1.32	1.44
	District x Location	3.88	4.22

**Figures in parenthesis are arcsine transformed values.

Table 2: Intensity of anthracnose of beans at various locations in Kashmir valley during 2011

District	Location	Per cent disease intensity*	
		Leaves	Pods
Anantnag	Larnoo	21.17 (4.60)**	33.38 (5.77)
	Danter	9.78 (3.12)	14.99 (3.876)
	Momin	10.26 (3.20)	17.44 (4.17)
	Mean	13.73 (3.70)	21.91 (4.68)
Bandipora	Chatibandi	10.89 (3.30)	18.37 (4.28)
	Bandipora	9.97(3.15)	15.07 (3.88)
	Sumbal	8.79 (2.96)	10.30 (3.20)
	Mean	9.88 (3.14)	14.58 (3.81)
Ganderbal	Batwani	9.23 (3.03)	17.37 (4.16)
	Yangura	11.82 (3.43)	22.88 (4.78)
	Zazun	8.75 (2.95)	11.51 (3.39)
	Mean	9.93 (3.15)	17.27 (4.15)
Shopian	Dradkralipora	9.87 ((3.14)	12.09 (3.47)
	Herman	9.03 (3.01)	10.52 (3.24)
	Sedav	10.08 (3.17)	13.18 (3.63)
	Mean	9.66 (3.10)	11.93 (3.45)
Overall mean	10.69 (3.26)	16.42 (4.02)	
$CD_{(P\ d^{*}0.05)}$	District	1.03	1.55
	Location	0.86	1.30
	District x Location	2.52	3.80

* Average of three sites. **Figures in parenthesis are arcsine transformed values.

Table 3: Morphological characters of fungus causing anthracnose of beans

Propagule	Size (μm)		Colour	Shape	Septation
	In culture	On host			
Conidia	9.5- 11.5 x 3.5-4.5(10.5x 4)	12-22.0 x 4-6(17.25 x 4.25)	Hyaline	Dumbbell, with obtuse ends	0
Setae	64-108 x 2-4 (84.22 x 3)	60- 120 x 5-6 (90 x 5.55)	Brown to Dark Brown	Cylindrical with pointed ends	0-3
Acervuli	140-320 (204.14)	175- 285 (270)	Black	Saucer Shaped	0

Plate 1: Categorisation of beanpods (open) infected with Anthracnose on 0-9 scale

With a view to assess the prevalence, disease incidence and intensity of disease, four districts viz., Anantnag, Bandipora, Ganderbal and Shopian, of Kashmir valley were surveyed during the crop season. The observations with respect to the disease incidence and intensity in different locations are summarised in Table 1 and 2 respectively. During the course of survey disease was recorded in all the locations surveyed showing its wide spread occurrence, the disease was observed on all aerial plant parts (Plate 2).

Disease incidence

On leaves

During the course of survey it was observed that on the under surface of leaves along the veins. The Highest disease incidence on leaves was recorded in district Anantnag, followed by district Ganderbal, Bandipora and Shopian. During the growing season the disease incidence ranged from 24.64 per cent to 53.79 per cent in district Anantnag, whereas it ranged from 27.02 per cent to 29.64 per cent, 23.51 to 28.15 per cent and 24.11 to 25.83 per cent in district Ganerbal, Bandipora and Shopian respectively.

The data further revealed that among the different locations surveyed, the disease incidence (53.79 per cent) on leaves was highest at Larnoo region of district Anantnag while the lowest disease incidence (23.51 per cent) was recorded at Sumbal of district Bandipora. Non-significant variation in disease incidence on leaves was noted for DradKralipora (24.48 per cent), Herman (24.11 percent) and Sedav (25.83 per cent) of district Shopian and Dantar (24.64 percent) of district Anantnag, which was statistically at par with SumbalofBandipora.

On pods

The disease incidence on pods was highest in distrct Anantnag (51.11 per cent) followed by Ganderbal (47.38 percent), Bandipora (32.96 per cent) and Shopian (28.99 per cent). The disease incidence on Common bean pods ranged from 32.70 to 82.14 per cent in district Anantnag, whereas, it ranged from 40.98 to 57.79 per cent, 25.65 to 43.51 per cent, and 26.90 to 31.88 per cent in district Ganderbal, Bandipora and Shopian, respectively. The data further revealed that that among the different locations surveyed the disease incidence (82.14 per

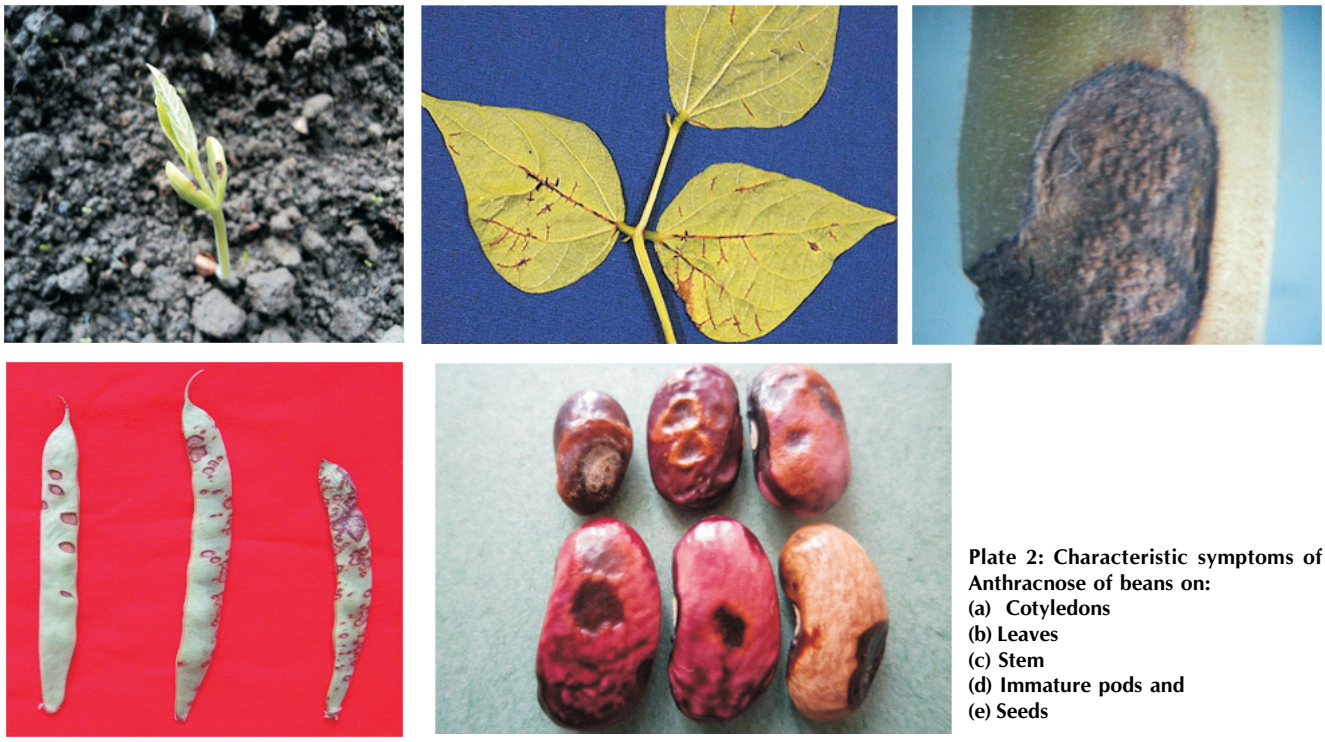

Plate 2: Characteristic symptoms of Anthracnose of beans on:
(a) Cotyledons
(b) Leaves
(c) Stem
(d) Immature pods and
(e) Seeds

cent) was highest at larnoo of district Anantnag, and lowest (25.65 percent) at Sumbal of district Bandipora which was statistically at par with Herman (26.90 per cent) region of district Shopian.

Disease intensity

During the course of survey it was observed that the disease was prevalent in all the bean growing areas of valley in varying intensities depending upon the climatic conditions of the location and the cultural practices followed by the farmers. The disease severity on leaves during the crop season was highest in district Anantnag (13.73 per cent) followed by Ganderbal (9.93per cent), Bandipora (9.88 per cent) and Shopian (9.66 per cent) respectively, though the districts Shopian, Ganderbal and Bandipora were statistically at par. Disease severity on leaves varied from 9.78-21.17 per cent in Anantnag while as it ranged from 8.79 to 10.89 per cent, 8.95 to 11.82 per cent and 9.03 to 10.08 per cent in district Bandipora, Ganderbal and Shopian, respectively. Disease intensity was highest at Larnoo (21.17 per cent) area of district Anantnag, followed by Yangura (11.82 per cent) of Ganderbal, which was statistically at par with the Chatibandi of Bandipora. Disease intensity on pods ranged from 14.99 per cent to 33.38 per cent in district Anantnag whereas it ranged from. 10.30 to 18.37, 17.37 to 22.88 and 10.52 to 13.18 percent in district Bandipora Ganderbal and Shopian, respectively. Over all disease severity on pods was highest in district Anantnag (21.91percent), followed by Ganderbal (17.27 per cent), Bandipora (14.58 per cent) and Shopian (11.93 per cent) though the districts Bandipora and Shopian are statistically at par with each other. The data further revealed that the disease development increases with time reaching maximum at the pod development stage.

Morphology

Morphological studies of fungus were carried out both on infected host tissue and PDA culture media and the results are depicted in Table 3

On host

Microscopic observations of the fungus revealed that the fungus produced acervuli as its fruiting structures that rupture the host epidermis and bear setae at its surface. Under humid, wet conditions the fungus produces characteristic ooze (Plate 3). Microscopic observation revealed that acervuli were saucer shaped measuring 175-285 μm with an average of 270 μm. Inside the acervulus conidia are produced that measure about 12-22 x 4-6ìm with an average of 17.25 x 4.2μm. The acervuli were found to possess bristle like appendages called the setae measuring about 60-120 x 5-6μm with an average of 90 x 5.55μm.

On culture

Macroscopic characters

Macroscopic observation of the fungus revealed that 5-7 days of incubationat 24 $\pm$ 1ºC, colony of the fungus on PDA medium appeared as grey when young and became dark black having very compact mycelial growth at late stage. The acervuli were formed after 10 days on the culture first in the centre, then in rings across the colony with salmon coloured ooze extruding from them. The acervuli were found to possess dark coloured bristle like projections called setae on the surface feature characteristics of this fungus.

Microscopic characters

Morphological studies of fungus on culture are presented in Table 3 (plate 4). Persual of the data revealed that conidia were hyaline, cylindrical, unicellular with obtuse ends, narrow

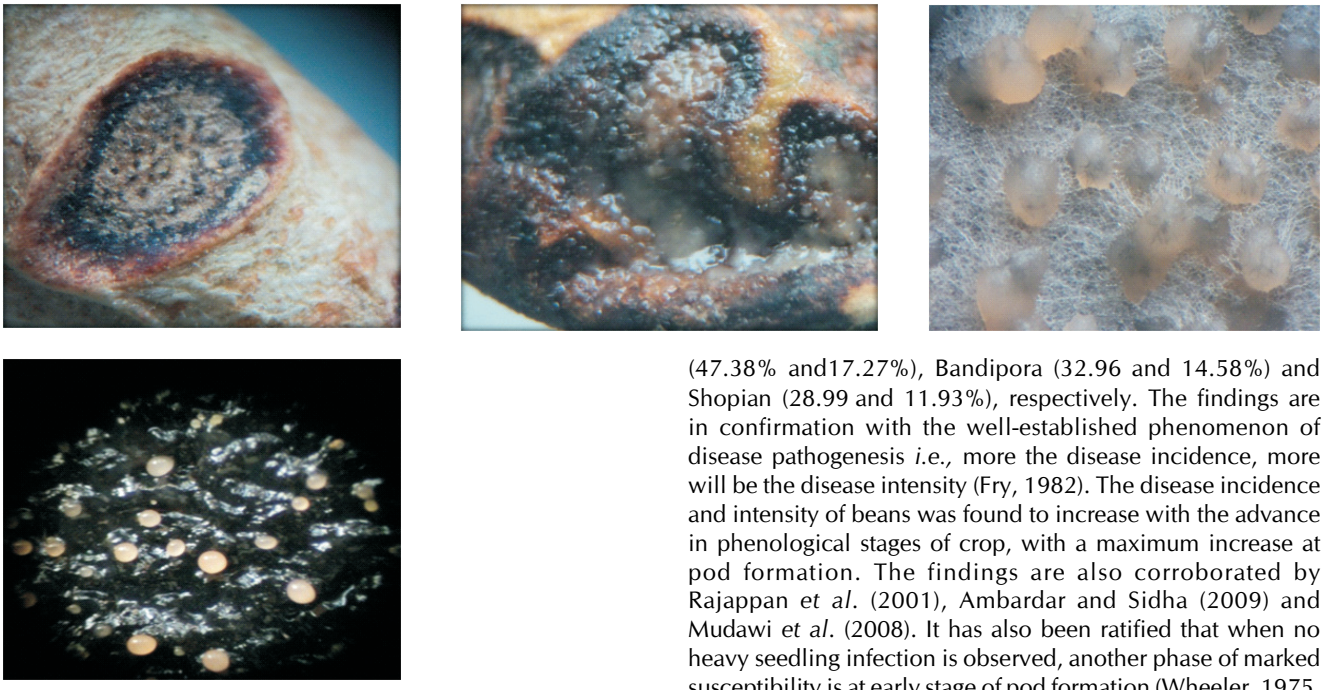

Plate 3: Morphological characters of *Colletotrichum lindemuthianum*
(a)Acervuli bodies on host; (b) Ooze on host
(c)Acervuli on culture (Under stereoscopic microscope)
(d)Ooze on culture (40x)

and truncate base, measuring about 9.5- 11.5 x 3.5-4.5μm with an average of 10.5 x 4μm. All the spores were without septa and uni-nucleate. Black acervuli were produced on 10 days old culture measuring about 140-320 μm with an average of 204.14μm. Setae were light brown to dark brown in colour measuring about 64-108 x 2- 4μm with an average of 84.22 x 3μm with 0-3 septa and few per acervuli.

DISCUSSION

The anthracnose of beans occurs in mild to severe form in every bean growing area of Kashmir valley, causing considerable losses (Khan *et al*., 2009). Establishing the status is pre-requisite to decide the adoption of disease management strategies. The objective was achieved in the present study by undertaking surveys in four commercially important bean growing districts of Anantnag, Bandipora, Ganderbal and Shopian of valley. It was observed that bean anthracnose was present in all the locations in varying proportions. The disease however, varied location to location due to difference in micro- and macro-climatic conditions.

During the course of survey incidence and intensity of bean anthracnose in the valley ranged from 23. 51 to 82.14% and 8.75 to 33.38%, respectively. Highest mean disease incidence and intensity on leaves, ranged from 34.81 % and 13.73% was recored in district Anantnag followed by distrct Ganderbal (27.90 and 9.95 %), Bandipora (25.43 and 9.88 %) and Shopian (24.80 and 9.66%), respectively. Least disease incidence and intensity of 24.80 and 9.66% was recorded in Shopian. Similarly, maximum disease incidence and intensity on pods was observed in district Anantnag (82.14 and 33.38%) followed by Ganderbal (47.38% and17.27%), Bandipora (32.96 and 14.58%) and Shopian (28.99 and 11.93%), respectively. The findings are in confirmation with the well-established phenomenon of disease pathogenesis *i.e.,* more the disease incidence, more will be the disease intensity (Fry, 1982). The disease incidence and intensity of beans was found to increase with the advance in phenological stages of crop, with a maximum increase at pod formation. The findings are also corroborated by Rajappan *et al*. (2001), Ambardar and Sidha (2009) and Mudawi *et al*. (2008). It has also been ratified that when no heavy seedling infection is observed, another phase of marked susceptibility is at early stage of pod formation (Wheeler, 1975. Gupta and Mathew, 2005). Increase in the disease severity with age may also be attributed to increase in susceptibility at pod development stage (Mudawi *et al*., 2008).

Maximum disease incidence and intensity on leaves and pods was recorded at Larnoo (53.79 and 82.14%, 21.17 and 33.38%) of district Anantnag follo - wed by Yangura (29.64 and 57.79%, 11.82 and 22.88%) of district Ganderbal, whereas lowest incidence and intensity was recorded at Sumbal of Bandipora (23.51%; 25.65% and 8.79%; 10.30%). The variation in disease incidence and intensity observed may be attributed to differences in the environmental factors, high dependence on farm saved seeds, exchange of diseased seeds among farmers, crop care practices, type of cultivar and the cropping pattern (David *et al*., 2000; Opio *et al*., 2001, Nkalubo *et al*., 2007).

Adoption of different in crop cult-ivation practices under diverse topographic locations with moist, cool climate, low night temperatures coupled with the race variability of the pathogen may also be deemed as feasible factors for disease variation (Pastor -Corrales, 1995; Bassenezi *et al*., 2001; Opio *et al*., 2006). While studying the incidence of bean anthracnose.

Morphological studies were carried out both on the infected host tissue and PDA media. Colony colour of fungus was grey when young and later on became dark black having very compact mycelial growth. Conidia were hyaline, cylindrical, unicellular with obtuse ends and narrow and truncate base measuring about 12-22 x 4-6 μm with an average of 17.25 x 4.2 μm on host and 9.5- 11.5 x 3.5-4.5 μm with an average of 10.5 x 4 μm. All the spores were without septa and uninucleate. The findings about spore-dimensions on culture are in consonance with those of Wijesekara and Agarwal (2006) and Khan *et al*. (2009). Acervuli were produced on 10 days old PDA culture, first in centre then in rings across the colony with salmon coloured ooze exuding from them. These findings

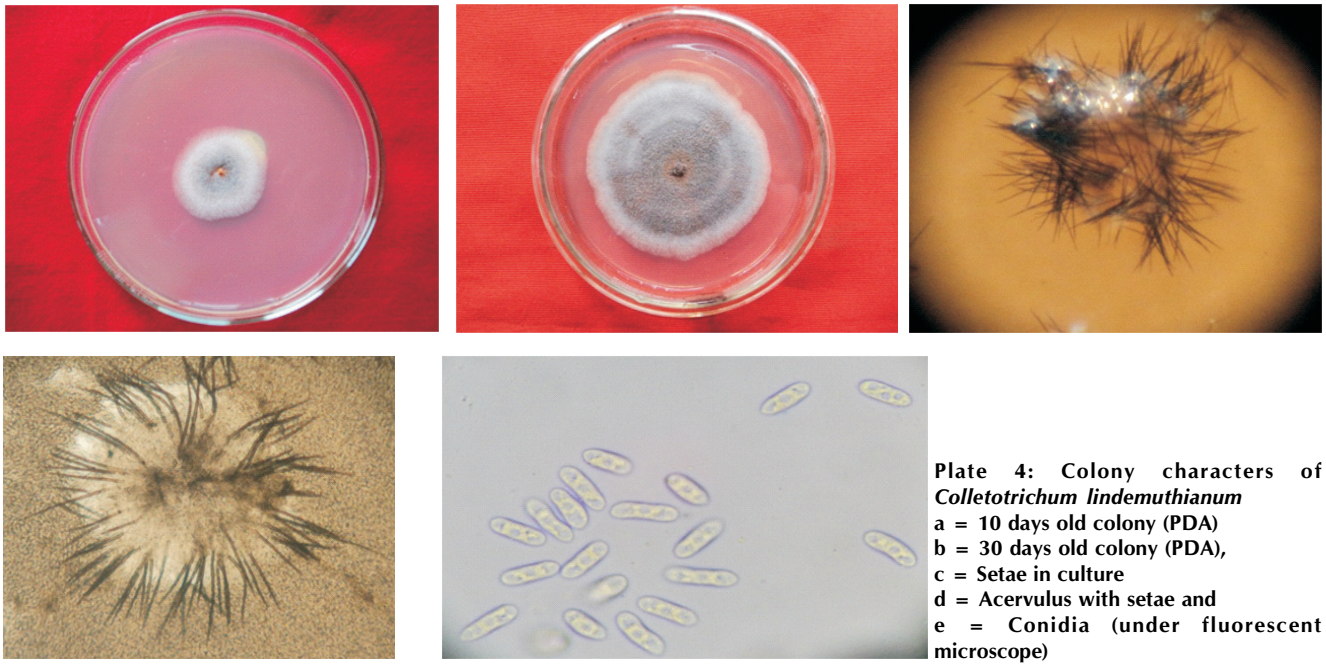

Plate 4: Colony characters of *Colletotrichum lindemuthianum*
a = 10 days old colony (PDA)
b = 30 days old colony (PDA),
c = Setae in culture
d = Acervulus with setae and
e = Conidia (under fluorescent microscope)

are scientifically substantiated by Khan *et al*. (2009). Adhikary *et al*., 2013 also reported the fungus *Colletotrichum gloeosporioides* causing mango anthracnose sporulates abundantly with maximum fruiting bodies in the centre of plate profuse mycelial growth towards periphery. The dimensions of acervuli ranged from 175-285 μm with an average of 270 μm on host. In artificial culture the dimensions ranged from 140-320 μm with an average of 204.14 μm. The acervuli possessed on the surface dark coloured bristle-like projections called setae, a conspicuous characteristic of this fungus. Setae were light brown to dark brown in colour measuring 60-120 x 5-6 μm with an average of 90 x 5.55 μm on host. In culture the corresponding dimensions were 64-108 x 2-3μm with an average of 84.22 x 3.0 μm, with 0-3 septa and few per acervuli. Similar findings have also been put forth by Kulshrestha *et al*. (1976).

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AIM & SCOPE

The journal aims to publish original peerly reviewed/ refereed research papers/reviews on all aspects of life sciences.

SUBMISSION OF MANUSCRIPT

Only original research papers are considered for publication. The authors may be asked to declare that the manuscript has not been submitted to any other journal for consideration at the same time. Two hard copies of manuscript and one soft copy, complete in all respects should be submitted. The soft copy can also be sent by e-mail as an attachment file for quick processing of the paper.

FORMAT OF MANUSCRIPT

All manuscripts must be written in English and should be typed double-spaced with wide margins on all sides of good quality A4 paper.

First page of the paper should be headed with the title page, (in capital, font size 16), the names of the authors (in capitals, font size 12) and full address of the institution where the work was carried out including e-mail address. A short running title should be given at the end of the title page and 3-5 key words or phrases for indexing.

The main portion of the paper should be divided into Abstract, Introduction, Materials and Methods, Results, Discussion (or result and discussion together), Acknowledgements (if any) References and legends.

Abstract should be limited to 200 words and convey the main points of the paper-outline, results and conclusion or the significance of the results.

Introduction should give the reasons for doing the work. Detailed review of the literature is not necessary. The introduction should preferably conclude with a final paragraph stating concisely and clearly the aims and objectives of your investigation.

Materials and Methods should include a brief technical description of the methodology adopted while a detailed description is required if the methods are new.

Results should contain observations on experiment done illustrated by tables and figures. Use well known statistical tests in preference to obscure ones.

Discussion must not recapitulate results but should relate the author's experiments to other work on the subject and give their conclusions.

All tables and figures must be cited sequentially in the text. Figures should be abbreviated to Fig., except in the beginning of a sentence when the word Figure should be written out in full.

The figures should be drawn on a good quality tracing/ white paper with black ink with the legends provided on a separate sheet. Photographs should be black and white on a glossy sheet with sufficient contrast.

References should be kept to a minimum and listed in alphabetical order. Personal communication and unpublished data should not be included in the reference list. Unpublished papers accepted for publication may be included in the list by designating the journal followed by "in press" in parentheses in the reference list. The list of reference at the end of the text should be in the following format.

1. **Witkamp, M. and Olson, J. S. 1963.** Breakdown of confined and non-confined Oak Litter.*Oikos*. **14**:138-147.
2. **Odum, E.P. 1971.** *Fundamentals of Ecology*. W. B. Sauder Co. Publ. Philadelphia.p.28.
3. **Macfadyen, A.1963.** The contribution of microfauna to total soil metabolism. In:*Soil organism*, J. Doeksen and J. Van Der Drift (Eds). North Holland Publ. Comp., pp 3-16.

References in the text should be quoted by the **author's name and year** in parenthesis and presented in year order. When there are more than two authors the reference should be quoted as: first author followed by *et al.*, throughout the text. Where more than one paper with the same senior author has appeared in on year the references should

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