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Exploration of Different Important Diseases of *Ber* (*Zizyphus mauritiana*) in West Bengal

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ABSTRACT

Ber is a perennial, tropical fruit crop belongs to the family Rhamnaceae. Fruits are used in dyeing silk and trees used for rearing of lac insect and other medicinal purposes. The yield of the crop is hampered due to various biotic stresses. Therefore a basic effort was made for gathering the knowledge about the various disease symptoms and their causal agents to develop efficient management strategies of the specific disease problem. Disease specimens were collected throughout the year from different parts of the state and the associated pathogens were isolated following standard laboratory techniques. The characteristic symptoms of these diseases were studied for identification of the related pathogens. Detailed cultural and microscopic studies of these causal agents were also described with macroscopic and microscopic photographic support. The major disease of *Ber* recorded were- black mildew (80-90%), fruit and leaf canker (50-100%) and powdery mildew (30-40%), the rust (25-30%), black leaf spot (70-80%), anthracnose (60-70%) while leaf spot, algal rust were recorded to be the minor diseases based on disease incidence. All the pathogens were grown in different media *in vitro* and their radial growth were noted at a particular days of interval.

Keywords *Ber*, Causal agents, Disease symptom, Media, Radial growth.

INTRODUCTION

Among the minor/under-utilized fruits, *Ber* (*Zizyphus mauritiana, Z. jujube*) is one of the most important fruit crops of tropical and subtropical region in India. This fruit crop is grown in semi arid regions of Haryana, Punjab, Rajasthan and other parts of India. Indo Malaysian region of South East Asia is regarded as center of origin of the crop. *Ber* is one of the economically important fruit crops. Area under *Ber* cultivation as per current data is 51,000 ha and production is 542,000 MT in India (Anonymous,2019-2020). *Ber* is known as Poor Man's Fruit or King of Arid Fruits.

Ber suffers from various diseases. Yuan *et al.* (2009) have reported various fungal diseases during their research work among them powdery mildew *Oidium zizyphi* or *Oidium erysiphoides* f. sp *ziziphi*,

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Table 1. Different locations of samples collection.

	District	Location	Latitude and longitude
1.	North 24 parganas	Mandouri	22°57' N and 88°20' E
2.	Nadia	Haringhata	21.5° N and 85° E, altitude 11.7 m above MSL
3.	Cooch behar	Cooch behar	26.3357° N, 89.4459° E
4.	Alipurduar	Damanpur	26.4919° N, 89.5271° E

Table 2. Composition of different media.

Media	Composition
1. Potato dextrose agar	Peeled potato-200g, Dextrose-20g, Agar agar-20g, Distilled water-1000ml, pH at 25°C.- 5.6±0.2
2. Carrot decoction agar	Carrot-200g, Dextrose-20g, Agar agar-20g, Distilled water-1000ml, pH at 25°C.- 6.5±0.2
3. Oat meal agar	Oat meal-60g, Agar agar-12.5g, Distilled water-1000ml, pH at 25°C.- 7.2±0.2
4. Host leaf extract	Fresh ber leaf- 200g, Dextrose-20g, Agar agar-20g, Distilled water-1000ml, pH at 25°C.- 6.6±0.2
5. Czapek dox agar	$NaNO_3$/NH_4Cl/KNO_3-2.00g/1.257g/2.379g, K_2HPO_4-1g, $MgSo_4$,$7H_2O$-0.5g, KCl-0.5g, $FeSO_4$,$7H_2O$-0.01g, Sucrose-30g, Agar agar-15g, Distilled water-1000ml, pH at 25°C.- 6.8±0.2

anthracnose (*Colletotrichum gloeosporioides*) *Glomerella cingulata* perfect stage were prominent. 14 different fungal organisms on various *Zizyphus species* were reported; out of which 12 organisms were reported to be occurred on *Ber* fruits causing fruit rots and other symptoms. In this research experiment we have tried to isolate the associated pathogens from infected disease samples, to report some major diseases of *Ber* and to identify the pathogens by comparing to the previous work of the researchers.

MATERIALS AND METHODS

The materials and methods followed in different experiments are described below.

i) Site selection for collection of disease samples: The samples of different diseases were collected from different location of West Bengal of *Ber* variety, BAU-1. Locations from where samples were collected are sited in Table 1. BAU-1 is very much susceptible to any rotting or leaf spot pathogen and most of the disease samples were collected from that variety.

ii) Media preparation:

For isolation and *in-vitro* radial growth studies of the pathogens, PDA medium was used. This medium acidic in nature with low carbohydrate. Low carbohydrate causes stress condition for the fungi and provokes spore production. Fungi prefer slightly acidic pH, so pH was maintained at 6.0-6.5. For fungal growth analysis different artificial media were utilized. The composition of these media has been described in the table later. Antibacterial antibiotic Chloramphenical @ 25 mg/lit of media was used to prevent the contamination with bacteria during isolation and purification of the fungal pathogen. Concentration of the applied antibiotic was 25 ppm (Table 2).

iii) Isolation of causal organisms: For isolation of causal organisms from the collected disease samples, some steps were followed.

a) Small triangular or square shaped sections were cut containing some infected and partly healthy portion with scissor, then the square sections were washed in running tap water properly and surface sterilized in 70% ethyl alcohol or 0.1% mercuric chloride for 15 seconds under laminar air flow to avoid surface contamination. Surface sterilizing agents were washed away thoroughly by running sterilized distilled water.

b) These sections/pieces were transferred aseptically to the petriplates containing PDA and incubated at 28±2°C. After 5-7 days of incubation a white or grayish white and in some cases black mycelial growth was appeared from the surface of the section (Devi *et al.* 2018).

Table 3. Survey on some major and minor diseases of *Ber*.

Disease identified	Place of collection	Infected part	% of area effected in plant	Associated pathogens
Black mildew	Cooch behar	Leaf and fruit	80-90% fruit part and 40-50% leaf part	*Mitteriella* sp. and *Pestalotiopsis* sp.
Cankerous Infection	Mandouri farm, Nadia	Fruit and stem	50-100% fruit part & 20-30% on stem	*Nectria* sp.
Powdery mildew	Mandouri farm, Nadia	Leaf	30-40% of leaf part	*Oidium erysiphoides*
Rust of *Ber*	Mandouri farm, Nadia	Leaf	25-30% leaf part	*Phakopsora ziziphi vulgaris*
Anthracnose and fruit rot	Haringhata farm, Nadia	Fruit and leaf	60-70% leaf and 30-40% fruit part	*Gloeosporium* sp.
Black leaf spot of *Ber*	Haringhata farm, Nadia	Leaf	70-80% of leaf part	*Isariopsisindica* var. *ziziphi*
Leaf spot of *Ber* (Both side)	Gayeshpur, Nadia	Leaf	20-30% of leaf part	*Cladosporium* sp.
Leaf spot of *Ber* (Upper)	Gayeshpur, Nadia	Leaf	20-25% of leaf part	*Curvularia* sp.
Red rust of *Ber*	Mandouri farm, Nadia	Leaf	70-80% of leaf part	*Cephaleuros* sp.

iv) Purification of isolated fungal pathogens : For obtaining pure culture hyphal tip method was followed (Choi 1999). The growing tip of the hypha was observed under microscope and a tip of that hypha was taken and transferred to the medium aseptically and incubated at 28±2°C and then grown culture was shifted to the culture tube for future purpose. This method was followed from the previous work of Brown (1924).

v) Microscopic observation : Diseased samples were observed under microscope at different power of 5X, 10X, 40X and photos of various fungal structures were taken. Similarly from the fungal culture the spores were observed on glass slides under microscope by staining.

vi) Pathogenicity test : Freshly prepared pathogens cultures were inoculated into their receptive healthy hosts. Before inoculation wounds were made using needles. Control treatment was also maintained accordingly. The inoculated leaves and fruits were kept in petriplates and trays were covered with polyethylene sheets in order to create favorable condition for disease development and kept in the incubator at 28±1°C. After 15–20 days pathogens produced similar symptoms like before. Pathogens were reisolated from hosts which showed symptoms after incubation. Pathogenicity test were done based on Koch postulate.

vii) Measurements of fungal spore and spore photographs : At first standardization of ocular micrometer with stage micrometer was done . Each fungal isolate was taken into clean slides and then one drop of lactophenol was added on that. After teasing coverslips were used to cover the fungal culture and kept under microscope. Ocular micrometer was inserted inside the eye piece and length and breadth of the fungal conidia was measured. Infected disease samples and purified associated pathogen culture were studied and photographs were taken under ZIESS Axiocam microscope and different types of propagules were recorded and spore micrometry was also done to identify the causal agents.

viii) Evaluation of radial growth variation and colony morphology of different fungal isolates in different solid media :

This experimental concept has been taken from the previous work done by Sharma and Pandey (2010) Ikechi-Nwogu and Elenwo (2012). By cutting the fungal colony with cork borer, one block was transferred to the center of solid media in the petriplates. The radial diameter of the fungal colony growth was measured at 3DAI, 5DAI, 7DAI. Approximately at 7DAI all petriplates were covered fully by different fungal growth. After full growth of the fungi on Petriplates the colony morphology of different fungi were studied and recorded accordingly.

ix) Statistical analysis : Opstat data analysis tool was

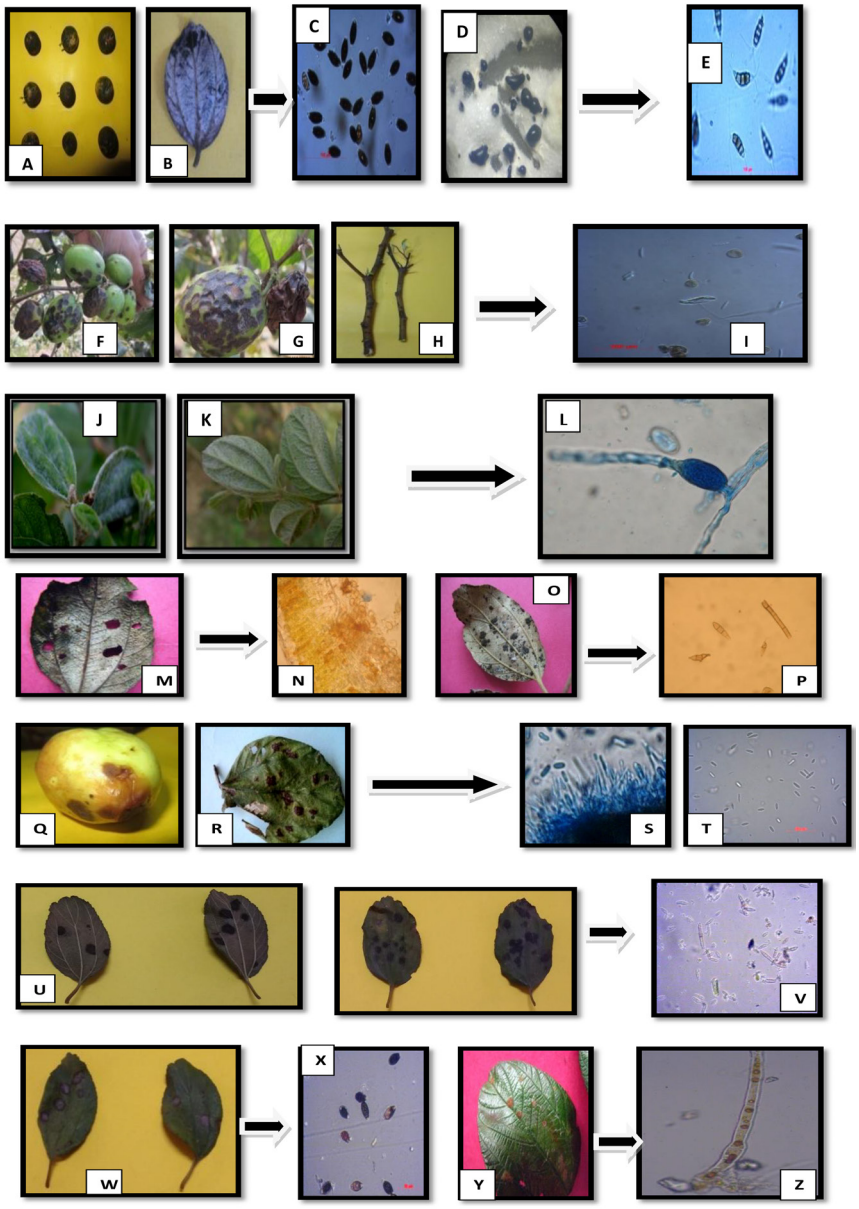

Fig. 1. [(A,B). Symptoms of Black mildew, (C)-Spores of *Mitteriella* sp., (D)-Spore ball of *Pestalpotiopsis* sp. (E)- Spores of *Pestalotiopsis* sp.] [(F,G,H)-Symptoms of Cankerous infection, (I)-Spores of *Nectria* sp.] [(J.K)-Symptoms of Powdery mildew upper and lower leaf surface respectively, (L)-Spores of *Oidium* sp.] .[(M)-Symptoms of Rust (N)-Spores of *Phakopsora* sp.] [(O)-Symptoms of black leaf spots, (P)-Spores of *Isariopsis* sp.] [(Q)-Symptoms of Fruit rot, (R)-Anthracnose, (S)-Fruit body Acervulus, (T)-Spores of *Gloeosporium* sp.] [(U)-Symptoms of Both side leaf spot, (V)-Spores of *Cladosporium* sp.][(W)-Symptom of Upper side leaf spot, (X)-Spores of *Curvularia* sp.] [(Y)-Symptom of Algal rust, (Z)-Sporangiophore of *Cephaleuros* sp.].

used for calculating Standard Error of Mean, Critical Difference at 5% and Coefficient of variation.

x) Percent disease incidence : Area of infected plant parts have been calculated based on the below mentioned formulae. Disease scale is from 1 to 9 based on leaf or fruit surface area showing symptoms.

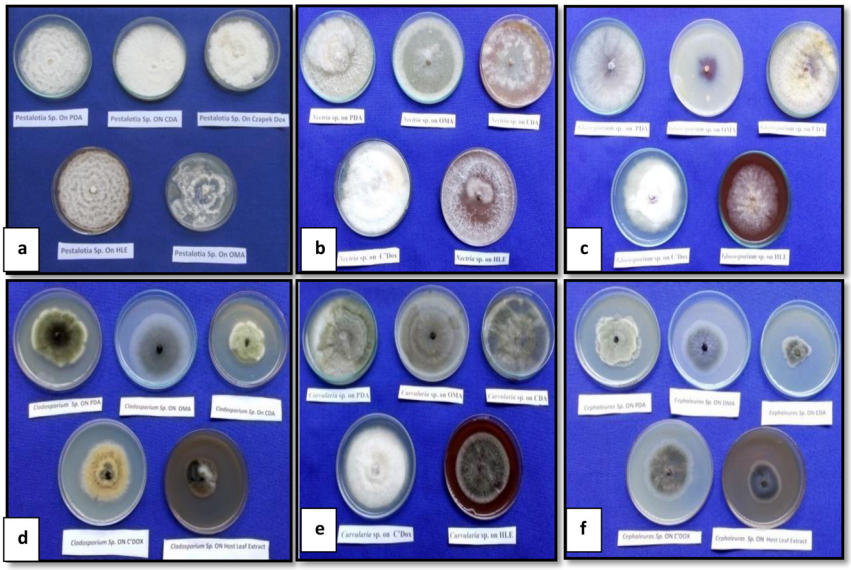

Fig. 2. Different pathogens growing in different laboratory media (PDA, OMA, CDA, Czapekdox, Host Leaf Extract) a. *Pestalotiopsis* sp. b. *Nectria* sp. c. *Gloeosporium* sp. d. *Cladosporium* sp. e. *Curvularia* sp. f. *Cephaleuros* sp.

$$\frac{\text{Part of fruits or leaves infected}}{\text{Total area of fruit or leaf observed}} \times 100$$

1 = No infection or tiny non spreading lessions
2 = ≤5% leaves or fruit surface effected
3 = Expanding lesions on 6-15% of leaves or fruits
4 = 16-30% leaves/fruits infected
5 = 31-45% leaves/fruits infected
6 = 46-60% leaves/fruits infected
7 = 61-80% leaves/fruits infected
8 = 81-90% leaves/fruits infected
9 = Whole leaves or fruits affected

RESULTS

i) Different disease symptoms :

All the disease samples collected during the course of investigation have been enlisted with their place of collection, infected plant part, percentage of area affected and associated pathogens and are presented in the Table 3.

ii) Description of symptoms and causal organisms :

a) Black mildew of *Ber* **:** Initial symptoms appear on the lower surface of the infected leaf during early winter (Nov-Dec). The infection appears as small, dirty black speaks on the leaf surface which quickly grows and coalesce to each other and ultimately covers the whole leaf surface. Symptoms are also appeared on developing fruits and in severe cases whole fruit surface is covered with black powdery growth of fungal spores Fig. 1 (A,B). Hoque *et al.* (2008) also studied with the same type of symptoms in *ber.* Associated fungal pathogens with this disease symptom are – *Mitteriella* sp. and *Pestalotiopsis* sp.

***Mitteriella* sp. :** Under microscopic study of black powder like fungal coating on the fruit and leaf surface, 3-celled conidia measuring 30.05×14.70 µm were observed. Both terminal ends of the conidia are hyaline and middle cells are dark brown. Both ends are flattened. Prominent guttulation can be observed at the middle cell. Middle cell is comparatively swollen

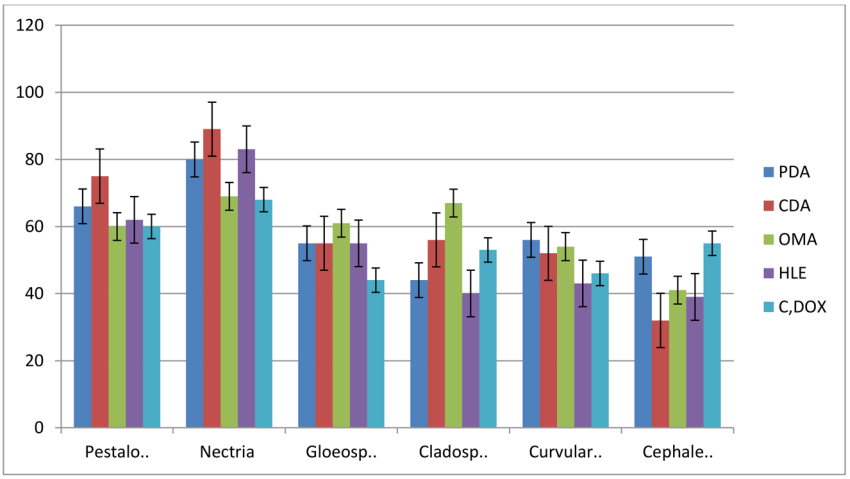

Fig. 3. Graphical presentation of comparative radial growth (mm) of different pathogens at 5DAI in different media. Error bars with standard error have been shown on the graph. X-Axis denotes media used, Y-Axis denotes radial growth of the pathogens.

and from that the terminal cell tapered towards both ends Fig. 1 (C).

***Pestalotiopsis* sp. :** From the spore ball of the fungus, the spores were identified Fig. 1 (D). Conidia are 4-5 celled, septate, measuring 18.84×7.25 µm. Both terminal cells are completely hyaline and middle cells are dark grey in color. Dark black colored prominent septa are present in multicellular conidia. At the anterior part 2-3 long appendages are present measuring 17.92–18.04 µm and at the posterior end single or sometimes double small appendages may be present Fig. 1(E). Gupta and Razdan (2010) first reported this pathogen as *Pestalotiopsis funerea* in *Ber* plant. With this reference we can identify this pathogen.

b) **Cankerous spot of ber :** The initial symptom recorded on the fruits surface during late winter (Jan-Feb) approximately at the time of maturity/harvesting. The infection appears as small, dark black, completely circular elevated lession (0.2-0.5cmdia) on the affected fruits which quickly grow and coalesce to each other. In some cases grayish black lessions are depressed, marked by raised prominent circular margin which can easily be defined. Numerous deep seated areas get merged with each other and cover the whole fruit surface. Small numerous white dot-like fungal structures can be seen on the spot of the fruits. In advanced stage of the disease the whole fruit become dry and mummified, severely affected fruits become reddish brown to black in color, which may hang on the shoot for long time. Symptoms can also be observed on branches and shoots of the plant where it over- summers. Circular cankerous lessions become visible on the infected stems also Fig. 1(F,G,H). Pathogen associated with the disease is identified as *Nectria* sp.

***Nectria* sp. :** Nectria sp. is the imperfect stage of *Fusarium* sp. Sickle shaped macro conidia measuring 21.37×4.25µm was clearly visible. Conidia are 7-8 septate, hyaline having pointed end and some cases ends are slightly curved Fig. 1(I).

c) Powdery mildew of *Ber* : Appearance of white patches on the surface of infected part can easily be observed, which eventually covers the whole surface of the young twigs and leaves Fig. 1 (J, K). With the advancement of the disease, infected tissues become shriveled. In course of time whitish conidial mass changed to pale white to light brown or dark brown patches in the month of February–March. On the infected fruits powdery growth/spots are easily visible, which grows quickly and covers the whole surface of the fruit. Symptoms of the powdery mildew of *Ber* was also described by Kapur *et al.* (1975), Kumar

Table 4. Measurement of spore size of different pathogen on PDA .

Sl. No.	Pathogens studied	Average spore size: Ranges of length and breadth of conidia (µm)	Mean of length and breadth of conidia (µm)	Length of appendages (if any) µm
1	*Mitteriella* sp.	26.37-32.31×13-16.92	(30.05×14.70)	No
2	*Pestalotiopsis* sp.	11.92-24.56×5.27-9.64	(18.84×7.25)	15.01-26.92 (17.92), 12.19-28.69 (18.04)
3	*Nectria* sp.	15.73-27.09×3.1-5.96	(21.37×4.25)	No
4	*Cladosporium* sp.	7.29-23.43×3.65-4.26	(15.49×4)	No
5	*Curvularia* sp.	22.76-33.46×13.13-15.94	(28.69×14.16)	No
6	*Gloeosporium* sp.	5.03-6.7×1.4-2.76	(5.73×1.95)	No
7	*Isariopsis indica* var. *ziziphi*	17-42×8.5-10.2	(35×9.2)	No

et al. (1978), Jamadar *et al.* (2009) and identified the pathogen associated with this disease is *Oidium erysiphoides*.

Oidium erysiphoides **:** The fungal pathogen is an ecto-parasitic in nature. The pathogen colonizes on the epidermal layer of the host tissue. Conidiophores are upright, single and bear cylindrical hyaline conidia at the tip. Presence of swollen or bulbous dark colored basal cell at the base of conidiophores is the major characteristic feature of this powdery mildew pathogen Fig. 1 (L).

d) Rust of *Ber* : The characteristic symptom of the disease is appearance of small, circular to irregular, light brown, yellow spore masses mainly on the lower surface of the leaves which gives a rusty appearance. With the advancement of the disease fungal mass spread gradually and cover large area of the leaves Fig. 1(M). Upper surface of the affected leaves showed normal grey appearance. Disease was prevalent during the month of March. Such type of symptoms of Rust disease was also described by Quan-YuJie (2000) *Phakopsora ziziphi-vulgaris.*

Phakopsora ziziphi-vulgaris **:** Large number of round to oval unicellular, thick walled, brownish orange colored uredospores emerge from uredopustules after rupturing the epidermis. Spores are developed on the tip of the spore bearing structure Fig. 1(N).

e) Black leaf spot of *Ber* : Circular to irregular, variable in size of black spot can be seen on the abaxial or dorsal side of the affected leaf. These spots give a sooty, tuft-like appearance, spread till the leaf blade and gradually cover a large area Fig. 1 (O). The corresponding upper surface shows brown discoloration. Symptom is observed mainly in post rainy season (Sept-Oct) on lower surface of the leaf. Jamadar *et al.* (2009) also observed the same type of symptoms and confirmed that the associated pathogen is *Isariopsis indica* var.

Isariopsis indica **var. *ziziphi*** **:** Conidiophores are multi-septate, long, dark brown. Conidia are light brown, multicellular (3-4 celled) broader at middle while tapering towards the both end, straight or sometimes bent, measuring 17–42 × 8.5–10. 2µm Fig. 1(P).

f) Anthracnose or fruit rot of *Ber* : Small circular to irregular corky, rough dark reddish brown spots appear with yellowish margin (2-4 mm diameter) on upper surface. Water soaked lessions on fruits. At the center of the spot a depressed zone or sporulating zone Fig. 1 (Q,R). Infected areas of the fruit peel become discolored and rapidly loosen the tissues which give a characteristic rotting appearance. Often, in severe infection fruit become very hard and if we keep the fruit on a moist chamber in BOD for 2 days, the white fungal growth developed and become clearly visible. Due to cell wall degradation cell sap comes out from the infected fruits, the characteristic feature of the soft rot disease. By close observation numerous dot like fungal fruiting body can be seen on the fruit surface. Associated pathogen is *Gloeosporium* sp. (Gupta and Madaan 1977).

Table 5. Radial growth of the different pathogens at 5DAI and 7DAI.

Media	*Pestalotiopsis* sp.		*Nectria* sp.		*Gloeosporium* sp.		*Cladosporium* sp.		*Curvularia* sp.		*Cephaleurus* sp.	
	5 DAI	7 DAI	5 DAI	7 DAI	5 DAI	7 DAI	5 DAI	7 DAI	5 DAI	7 DAI	5 DAI	7 DAI
PDA	66	89	80	90	55	83	44	67	56	77	51	73
CDA	75	90	89	90	55	90	56	80	52	78	32	53
OMA	60	73	69	90	61	90	67	80	54	90	41	63
HLE	62	89	83	90	55	69	40	53	43	60	39	54
C'DOX	60	75	68	90	44	72	53	75	46	68	55	68
SE(m)	1.29	0.483	0.87	0.14	0.537	1.48	1.062	1.10	1.20	0.95	1.25	0.68
CD at 5%	4.29	1.60	2.91	00	1.78	4.92	3.517	3.65	4.00	3.15	4.15	2.26
CV	3.46	1.00	1.95	0.28	1.73	3.18	3.537	2.68	4.17	2.21	4.99	1.89

***Gloeosporium* sp. :** Bullet shaped, thin walled, unicellular, hyaline conidia are produced at the top of upright, hyaline, multi-septate conidiophores Fig. 1(S,T). D.K. Misra, Rabidas P. Vijoy, Bauri F.K. (2007) also found this structure without any special structure of setae.

g) Leaf spot of *Ber* : Symptoms appear on both leaf surfaces. Dark black circular, regular spots can be seen on the upper surface of the leaves. Spots are isolated but sometimes they coalesce to each other. On the corresponding lower surface black colored circular spots were observed but on this side spots are intervenal. 2-5 spots were on lower surface measuring 1-3mm in diameter Fig. 1(U). Disease was not so severe and associated pathogen was confirmed as *Cladosporium* sp.

***Cladosporium* sp. :** Pathogen produces bi-celled, single septate, conidia. Conidia are darkly pigmented measuring 15.4 × 4 um length and breadth. Conidia may be present singly or sometimes in branch Fig. 1 (V).

h) Leaf spot of *Ber* (Upper surface) : Symptoms appear on the upper surface of the leaves. Oval to circular spots with dark brown to black prominent margin with ash colored center can be observed. Spots are conspicuously delineated, may vary from 2-5 in number on each surface Fig. 1(W). Black conidial mass can be seen on the spots. Associated pathogen is identified as *Curvularia* sp.

***Curvularia* sp. :** Conidiophores are erect, unbranched and septate. Conidia are 3-4 celled, barrel shaped or curved boat shaped measuring 28.69×14.16 µm. Two terminal cells are hyaline and middle cell is dark brown in color. Middle cell is larger than apical and basal cells. Conidia are having brown smooth wall Fig.1 (X).

i) Red rust of *Ber* : On the upper surface of the leaves reddish brown circular to irregular spots can be observed. They give a rusty appearance in advanced stage Fig.1(Y). Spots are measuring 2-4 mm in diameter and numerous in numbers. Similar type of symptoms was studied by Lim *et al.* (2003) on mangosteen plant and confirmed the causal agent as alga-*Cephaleuros virescens* causing algal spots on mangosteen. Crane (1994) also described with rust disease of carambola. Therefore, the associated pathogen may be identified as an algae *Cephaleuros* sp.

***Cephaleuros* sp. :** This is a common species of algae. Under microscopic study sporangia and sporangiophores were observed. Sporangiophores are long, 4-8 septate. Number of sporangia produce by the sporangiophore may vary from 5-8. Sporangia may easily detach from suffultory cell of the sporangiophore Fig. 1 (Z).

Measurement of fungal spores : Length and breadth of conidia and other fungal propagules are enlisted in Table 4.

Cultural characterization of pathogen isolates grown in different media : Five different media including synthetic, semi synthetic and natural

Table 6. Growth behavior of pathogens on PDA, CDA, Cz, OMA, HLE media.

Pathogens	PDA	CDA	OMA	HLE	Cz
Pestalotiopsis	White color Even, wavy growth	White, evenly grown, depressed	Very less growth, uneven, white	Slightly pinkish, even, wavy	Comparatively less growth, white, uneven
Nectria	Light pink, uneven, not smooth texture,	Less growth, uneven, light brown	Off white color, uneven growth	Radial growth, dirty white color, not smooth, uneven	Very vigorus growth, no pigmentation
Gloeosporium	Vigorus growth, evenly distributed, not smooth, purple pigmentation at the point of incubation	Highly dense growth, fluppy, yellow pigmentation	Comparatively evenly distributed, less growth, depressed, violet pigmentation at the point of incubation, mat like	Evenly distributed comparatively less growth	Uneven less growth, no pigmentation
Cladosporium	Uneven fluppy growth, dark olive green mycelia, actively growing part is white and center is black.	Comparatively less uneven fluppy growth, olive green	Mat like, light bluish growing mycelia and dark center	Dark brown pigmentation, at center white mycelia, fluppy	Uneven, orange yellowish pigmentation
Curvularia	Dense growth, olive green pigmentation, fluppy	Fluppy thin growth, light black	Evenly distributed, dense, mat like, blackish growth	Sparsely distributed, brownish black	Dense fluppy growth, no pigmentation. Milky white
Cephaleuros	Evenly distributed, white, thin mat like	Very less uneven growth, blackish	Very thin, blackish	Evenly grown, chocolaty brown	Mat like, comparatively higher growth, light black

media were used in this investigation and these are-Czapek dox agar media (C'Dox), Potato dextrose agar (PDA), Carrot leaf decoction agar (CDA), Oat meal agar (OMA) and Host leaf extract agar (HLA). *In-vitro* growth behavior and morphological variations of the pathogen and their colony growth were recorded and presented. In most of the cases isolates favor PDA media for *in-vitro* growth and development. *Pestalotiopsis* sp. grows well in all the media tested except on OMA where it grows abnormally without having any definite pattern of colony. Sajeewa *et al.* (2011) recorded the same nature and phylogeny of *Pestalotiopsis* sp. Fig. 2(a). *Nectria* sp. is a fast growing pathogen which grows well on all the five laboratory media tested Fig. 2(b). OMA was recorded good for growth of *Gloeosporium* sp. where the colony is fluppy with dense mycelia and profuse sporulation Fig. 2(c). For *Cladosporium* sp. OMA showed the best result. The dark colony with white mycelium showing the clear peripheral zone in OMA. Saltation was recorded on host leaf extract agar media where the colony growth of the pathogen was lowest. Sharma and Pandey (2001) studied the artificial colony of *Cladosporium* sp. and observed dark colored colony growth on PDA media Fig. 2(d). *Curvularia* sp. is comparatively weak pathogen and OMA and PDA were recorded to be the best. Fig. 2 (e). The *Cephaleuros* sp. is a weak pathogen and PDA was recorded as best Fig . 2(f).

Radial growth of different pathogens on different laboratory media has been presented graphically on Fig. 3. Radial growth of the pathogens was measured at 5DAI, 7DAI, the measurement of the radial growth has been presented in Table 5 and cultural characterizations have been described in Table 6.

DISCUSSION

The ***Ber*** is one of the important minor fruit crop prone to be attacked by numerous fungal and algal diseases.

The major newly reported disease is Black mildew caused by *Mitteriella ziziphina* (Hoque *et al,* 2008). Other diseases viz., cankerous infection (*Nectria* sp.), anthracnose (*Gloeosporium* sp.), powdery mildew (*Oidium erysiphoides*) and rust (*Cephaleuros* sp.) were also seemed to be devastating diseases of *Ber*. In this context the study on the characteristic symptoms of these diseases was done after collection of the various disease specimens from different part of the state. The symptoms of these diseases were found to be similar with the outlines of disease symptoms exerted by different scientists. All the pathogens of the above mentioned diseases were identified based on their etiology under microscopic study and previous workers' references as well as pathogenicity test. But in case of black mildew it was found that *Pestalotiopsis* sp. was identified as an infectious propagule from fungal culture instead of *Mitteriella ziziphina*. The isolated pathogens were allowed to grow in five different artificial media (PDA, CDA, OMA, HLE, C'DOX) to find out suitable media for each pathogen so that specific medium can be reported for easily isolation of the specific pathogen in laboratory condition from the infected part of the tree for further research purposes. Therefore at a specific day's interval (3 days, 5 days and 7 days) observation on radial growth of the each pathogen in each medium was taken carefully. As some of the pathogens took full plate growth at 7DAI, observations on radial growth of 5 DAI was taken into consideration for graphically presentation and to make a clear sense about the comparison of pathogens' preference of the media. It was found that most of the pathogens prefer CDA and PDA media and in few cases OMA while very less in HLE media. Hence from this study the basic idea was obtained about characteristic symptoms of the major and minor diseases of *Ber* in West Bengal along with their infecting agent and morphological structure and ultimately their suitable media for easily isolation.

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