Studies on leaf blight disease of medicinal plants (*Piper longum, Tylophora indica, Hibiscus subdariffa)* in lower gangetic plains of West Bengal

Abstract:

Herbal medicinal plants are in great demand for their use in food preparations, pharmaceuticals, cosmetic and beauty aids. But its availability is reduced every year due to attack of fungal disease i.e. leaf blight. Therefore an experiment was conducted to gather the knowledge about the future management aspect of this disease. Here three medicinal plants viz, *Piper longum, Tylophora indica and Hibiscus subdariffa* were planted and observed the disease throughout year. The characteristic symptoms of the disease along with causal organisms (*Colletotrichum gloeosporioides- Piper longum, Sclerotianum rolfsii- Tylophora indica, Fusarium sp- Hibiscus subdariffa*) were recorded. In the seasonal study, the lowest disease incidence and severity of the disease were observed in the month of August and higher in June. *Hibiscus subdariffa* showed less disease incidence (15.33%) and severity (6.11%) at initial stage (August) and 67.33% & 42.46% respectively at peak period (June). But *Piper longum* and *Tylophora indica* showed higher disease incidence and severity at the same times. All these pathogens were isolated in PDA medium from the infected parts of plants and confirmed their pathogenicity. The cultural characteristics along with their micrometric measurement of spores were recorded in four different media (PDA, CZA, OMA and MMA). *Colletotrichum gloeosporioides* was found to grow better in PDA (9.0 cm) along with high dry weight (1.47g) and lowest growth in maize meal medium (4.8cm). In *Sclerotium rolfsii***,** highest radial growth (9.0cm) and dry weight (1.48g) were observed in maize meal medium but no growth in Czepek'dox. *Fusarium* sp showed highest radial growth (8.3 cm) and dry weight (1.34 g) in czepek dox and lowest in maize meal agar medium (4.4cm).

Key words: Disease incidence, Disease severity, *Hibiscus subdariffa*, Leaf blight, *Piper longum, Tylophora indica.*

Introduction:

"Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell" (Lai and Roy, 2004; Tapsell *et al.*, 2006). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

More than 30,000 plant species of medicinal importance are grown all over the world. Extensive cultivation of these plants has increased the pathological problems. The diseases of these plants and their intensity have increases to a great extent. An attempt to conserve and protect these plants presents a formidable challenge. Investigations to detect and evaluate the pathogens causing heavy losses are still incomplete and need high priority. Some of the medicinal plants are becoming extinct and suitable measures must be implemented so that this great wealth is not lost. According to the WHO report, at least 80% of the population of developing countries depends on traditional medicines for their health care.

India is one of the major exporters of medicinal plants. Ever increasing pressure on the natural resources has led to widespread deforestations and destructions of natural habit. Due to which around 40 plant species having medicinal properties are near extinction. If properly preserved India's biodiversity could yield millions in hard currency. "West Bengal exhibits a varied range of topography and agro-climatic conditions, which enormously contribute on its vegetation and floristic consumption. In spite of this, information on area under medicinal plants and production are not available" (Das *et al*, 2002). Cultivation of medicinal plants in West Bengal is yet to take a noble shape. West Bengal government has recommend some medicinal plants like Aswagandha, Sarpagandha, Senna, Tulsi, Pipul etc for commercial cultivation in different zones. But the several biotic (Fungi, Bacteria, virus, mycoplasma, nematode) and abiotic (Nutrient deficiency) factors limit the production of these crops as well as the quality of the produces and acceptability to the market.

The biotic factors causing different important diseases of these crop plants viz, root rots, cankers, wilts, leaf spots, scabs, blights, anthracnose, rusts, mildews, smuts, mosaics, yellows, root knots, etc are characterized by their presence in the tissues of these plants and

indicate that they are probably the cause of the disease. Proper diagnosis and management of these diseases are very essential to protect these plants. Therefore in this present dissertation, attempts had been made to study the important diseases of some medicinal plants (Leaf blight) with the following objectives:

- To characterize the disease symptoms of leaf blight in three medicinal plants- *Piper longum, Tylophora indica* and *Hibiscus subdariffa*
- To study on the seasonal incidence of leaf blight in these plants throughout the year.
- To isolate and characterize the pathogens causing leaf blight disease and their pathogenicity test following Koch's postulate.

Materials and Methods:

The medicinal plants for the experiment were planted at the In-check Farm, BCKV, Kalyani, Nadia and the survey studies was done from the month of August, 2019 to June, 2020.

Disease conditions in the plants were recognized according to the symptoms produced by the pathogens. The plants were carefully noticed to describe the characteristic symptoms of the disease produced on the leaves of three different medicinal plants (*Piper longum, Tylophora indica* and *Hibiscus subdariffa).*

To determine the disease incidence and severity of leaf blight disease in these medicinal plants observations were taken at 15 days intervals from the month of August, 2012 to June, 2013. For percent disease incidence no. of infected leaves and total no. of leaves (Infected+ healthy)/plant in a plot were counted and for percent disease index, the percentage of infected area per leaf / plant was recorded in each tagged plant through visual observation in each plot and were rated on a 0-5 scale, where 0= healthy leaves; 1= 1 - 10% leaf area infected; 2= 11 - 20% leaf area infected; 3= 21 - 40% leaf area infected; 4= 41-60% leaf area infected and 5= above 61% leaf area infected. Disease incidence and disease severity were calculated following the formulae below:

$$\textbf{Disease incidence} = \frac{\text{No. of infected leaves per plant}}{\text{Total no. of leaves per plant}} \times 100$$

$$\textbf{Disease severity (PDI)} = \frac{\sum \text{Numerical ratings}}{\text{Total No. of units (leaves) observed /plant} \times \text{highest rating}} \times 100$$

Isolation of the fungi causing foliar disease on medicinal plant:

The leaves which showed some spots or lesions were collected from the field and brought to the laboratory for isolation of the fungi causing diseases on them.

Isolation was carried out in a sterilized zone of the laminar air flow. The diseased specimens already washed with tap water were taken and with the help of a sterilized scissor, small pieces of the leaf were cut into small pieces which contained the diseased portion as well as the healthy tissue. The pieces were dipped in $HgCl_2$ solution (0.1%) for 1 min. and then rinsed for three to four times thoroughly with sterile distilled water to remove the excess solution. With the help of a sterilized forceps, each piece was placed aseptically on the solidified PDA in sterilized plates depending upon the diseased specimen. About 3-4 such pieces were placed on each plate maintaining some distance from each other and the Petriplates were incubated at 28 $\pm$ 1^0C. After 5 days, the growing fungus was examined under micro-scope for sporangial production. The isolates were maintained on potato dextrose agar medium. All the isolates were preserved at 5 ^{0}C. Sub cultures were made at 15 days intervals.

PATHOGENICITY TEST:

For artificial inoculation, at first the culture of inoculum was prepared in PDA media in the Petri plate. After getting the culture of the test fungi, spore suspension was prepared with sterile distilled water. Spore suspension and sterile distilled water were sprayed on few healthy plants separately and then covered by the poly-propylene packet to maintain the humidity as well as the favourable condition for disease development. After 15-20 days of inoculation on the test plants, disease symptoms were developed. The diseased leaves were collected and again re-isolated the pathogens to compare with the previous isolated pathogens and to get confirm about the disease causing pathogens.

Cultural and morphological characteristics of these pathogens:

To study the colony characters of different fungi in different media, four types of media namely PDA, Oat meal, Maize meal and Capek's dox were used.

After preparation of these media were sterilized in an autoclave at 15 p.s.i for 15 minutes. Then these media were poured into sterilized Petri plates in an aseptic zone of laminar air flow. Plates were allowed to cool and solidify the media. After solidifying of each medium the plates were inoculated by fungal disc. Fungal disc was taken from 4 days old fungal culture. After inoculation, plates were kept in BOD incubator at 28± °C. Different changes of fungal colony in different media were recorded every day up to 10 days. The growth of different fungi in different media was also measured every day up to 10 days.

The slides of the selected fungal cultures or colony were prepared in order to study the fungal morphology such as the characteristics of the hyphae, spores, etc. for easy identification of the fungal species infecting a particular specimen. The prepared slides were observed under Phase-contrast microscope using ocular and stage micrometer.

Measurement of dry weight of pathogens in different media:

To study the dry weight of different fungi in different liquid media, four types of liquid media were prepared same as mentioned previous without agar.

After preparation of different liquid media, it poured into conical flask and sterilized in an autoclave @15 p.s.i for 15 minutes. Then autoclaved media were allowed to cool in laminar air flow and inoculated by fungal disc. Fungal disc was taken from 4 days old fungal culture. After inoculation, the flasks were kept in BOD incubator at 28± °C for ten days. After 10 days, fungal mycelial mat was removed from the flask and kept in drier at 50 °C for 48 hours. After drying, the weight of mycelial mat was recorded. Three replications were maintained for each pathogen in each different media.

Result and discussion:

The findings obtained from the experiment are presented below preciously. At first leaf blight disease of three medicinal crop plants were identified in the field and its characteristic symptom were elaborately described.

Leaf blight disease symptom of *Piper longum* caused by *Colletotrichum gloeosporioides*:

Dark chocolate, irregular and angular spots occurred on the leaves. The spots were surrounded by yellow halo which extended from the margin and any part of the leaf. No spots were found on the youngest leaves.

The above described symptom of leaf blight disease is almost similar with Kendre *et al* (2017) who described that elliptic to oblong spots of variable size appears on both surfaces

of leaves, but more on the upper. In case of very severe infection, most of the leaves may dry up; presenting a bad appearance in the field, in such case the loss may be more than 50 per cent and also noted that the disease was caused by *Colletotrichum gloeosporioides* in field condition.

Leaf blight disease symptom of *Tylophora indica* caused by *Sclerotianum rolfsii:*

Symptoms first appeared on the tips of the leaves where yellow water soaked discoloration occur. Later, the margins of the leaves also started yellowing uniformly and spread gradually downwards and inwards of the leaves until the entire leaf blades turned yellow and ultimately wilted and died. But the main characteristic symptom of this disease is burning appearance of the tips of the leaves. Irregular deep brown spots also appeared on the leaves. This type of symptom had not yet been observed in any literature. So, this might be a record of new disease of *Tylophora indica*. However, further confirmation of results is required before final conclusion.

This type of symptom in Antamul (*Tylophora indica)* was noticed by Sarkar *et al* 2017 and also stated that the disease was caused by *Sclerotianum rolfsii*.

Leaf blight disease symptom of *Hibiscus subdariffa* caused by *Fusarium sp*.

Dark chocolate, irregular shaped spots without any halo and gray spots were present on the leaves. Spots tend to coalesce. Symptoms could be visible on both the surfaces of the leaves. The midribs of the leavers mostly escape from infection. In severe cases, the spots coalesce together causing blighting of the leaves from the base and increased upwards.

The similar type of symptoms of the disease was observed by Amusa *et al* (2001) in the tropical forest region of south-west Nigeria and reported that leaf blight symptoms were associated with infection by *Phyllosticta hibiscini* and stem blight with *Fusarium oxysporum*.

Disease incidence and severity of leaf blight disease of these medicinal plants (*P .longum, T. indica, H. subdariffa*) (August, 2012 – June, 2013):

The results revealed that the lowest and highest disease incidence and severity of the leaf blight disease were recorded during August-November (2012) and March- June (2013) respectively. The very lowest disease incidence and severity were observed in the month of August ranging from 15.33 to 22.37% and 6.11 to 10.33% respectively and highest disease incidence (67.33-78.66%) and severity (42.46-48.19%) in the month of June in these three medicinal plants. The lowest disease incidence (15.33%) and severity (15.33%) were recorded in *H. subdariffa* followed by *P .longum* with disease incidence (19.67%) and

severity (8.55%).But *T. indica* showed comparatively high disease incidence (22.37%) and severity (10.33%) in this period**.** The disease incidence and severity of this disease were gradually increased with increasing the time and reached to a peak during June. The highest disease incidence (78.66%) and severity (48.19%) were noted in *T. indica* followed by *P. longum* where disease incidence and severity were 73.39% and 45.41% respectively and comparatively lowest disease incidence (67.33%) and severity (42.46%) were observed in *H. subdariffa* (Table-1).

This type of result was observed by Alam (2004) who stated that leaf blight disease was appeared in the month of August and September with less disease incidence and severity in *Piper longum* but it appeared in severe form in comparatively dry weather in October and November and onwards .

Table no.1: Disease incidence and severity of leaf blight disease of three medicinal plants (*P .longum, T. indica, H. subdariffa)* throughout the year.

Months	Medicinal plants					
	P .longum		***T. indica***		***H. subdariffa***	
	% Dis. Inc	**% Dis. Index**	**% Dis. Inc**	**% Dis. Index**	**% Dis. Inc**	**% Dis. Index**
Aug,12	**19.67**	**8.55**	**22.37**	**10.33**	**15.33**	**6.11**
Sept,12	28.62	12.19	29.33	16.90	23.61	11.37
Oct,12	38.31	18.59	40.67	19.31	34.66	15.25
Nov,12	45.81	23.43	47.56	28.96	39.66	20.63
Dec,12	53.39	26.33	54.79	31.55	46.93	23.33
Jan,13	55.16	29.67	57.33	33.58	49.33	27.36
Feb,13	61.44	33.79	63.67	37.22	56.29	31.69
Mar,13	65.38	37.66	68.31	40.02	58.69	34.44

April,13	68.47	40.66	71.33	43.57	62.67	37.84
May,13	70.23	42.70	73.29	45.29	64.67	39.48
June,13	**73.39**	**45.41**	**78.66**	**48.19**	**67.33**	**42.46**

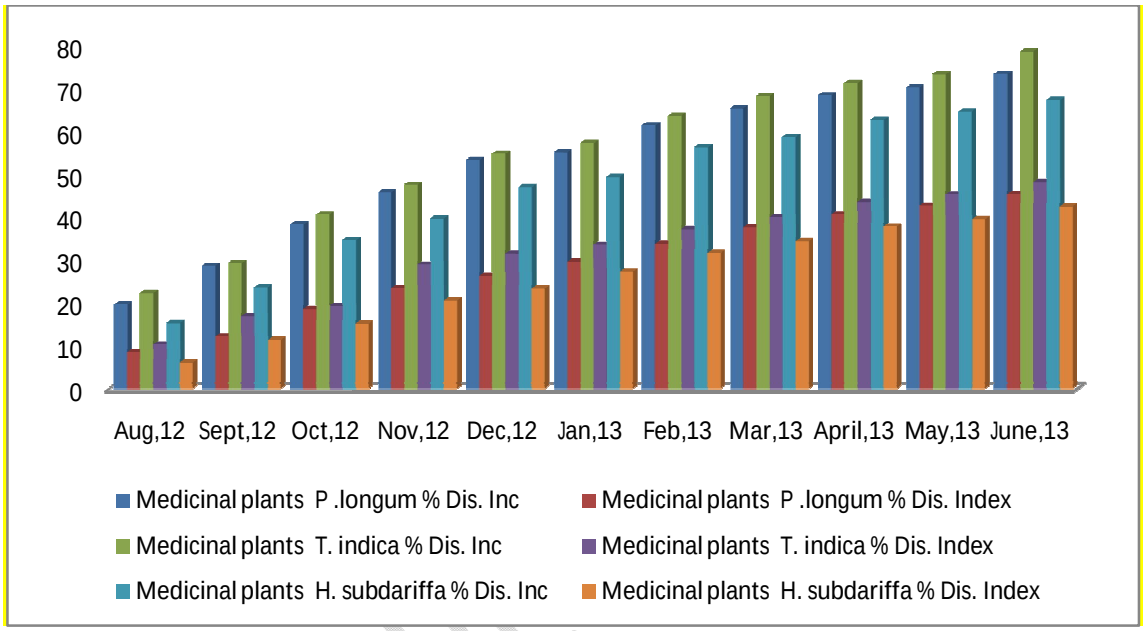

Fig1. Disease incidence and severity of leaf blight disease of three medicinal plants (*P .longum, T. indica, H. subdariffa)* throughout the year.

Cultural behavior of the pathogens causing leaf blight disease in three different medicinal plants:

The colony characters of these pathogens were studied after 5 days of inoculation in PDA media by visual observation and under Phase – Contrast Microscope (Table - 2).

Table no.2: Important cultural characteristics of the pathogens:

Name of the Crop	Name of the Fungi	Colony Characters by visual observation and under Phase-Contrast Microscope
P. longum	*Colletotrichum gloeosporioides*	White mycelial growth, dense powdery mass (acervuli) are present on the growth.

T. indica	*Sclerotium rolfsii*	Very white fluffy growth over the leaf pieces.Tip of the hyphae looking as such to produce conidia like structures; mycelium was septate.
H. subdariffa	*Fusarium sp*.	Olivaceous green centre with fluffy growth over the leaf pieces.Produced micro-conidia which were boat-shaped and bicelled.

Pathogenicity test of the pathogens isolated from these medicinal plants:

Pathogenicity of leaf blight of *Piper longum, Tylophora indica* and *Hibiscus subdariffa* was tested on healthy potted plant following the method described in the chapter "methods and materials". Development of similar types of symptoms after isolation and re-inoculation with spore suspension of these pathogens confirmed the pathogenicity of the pathogen. After re-isolation in PDA media visual observations of the colony characters were made and presented in Table - 3.

Table no.3: Cultural behaviour of the re-isolated pathogens from the inoculated plants with the same pathogens:

Name of the Crop	Name of the causal organism	Colony Characters by visual observation
P. longum	*Colletotrichum gloeosporioides*	Dark gray – white fluffy colony; back of the media was completely black.
T. indica	*Sclerotium rolfsii*	Creamy-white dense fungal colony; back of the media was cream colored.
H. subdariffa	*Fusarium sp.*	Concentric fungal colony having pink to light brown centre and black borders. The borders were again surrounded by pink outlines. They had patches of white, slightly dense colony over them.

Radial growth of these pathogens in different media:

The fungus was allowed to grow in five different media such as Potato Dextrose Agar media (PDA), Czepek dox media (CZA), Oat meal agar media (OMA) and Maize meal agar media (MMA). Data was taken for ten days. The results (Table 4) showed that the highest growth of *Colletotrichum gloeosporioides* was observed in PDA (9.0 cm), lowest growth in maize meal (4.8). In case of *Sclerotium rolfsii***,** highest growth observed in maize meal media (9.0 cm) but in Czepek'dox media pathogen can't grow. In case *Fusarium* sp, highest growth observed in czepek dox (8.3 cm) than other three media.

Table no.4: Radial growth of *Colletotrichum gloeosporioides*, *Sclerotium rolfsii* and *Fusarium sp* in different media:

Different media	**Radial growth of different pathogens in different media after ten days of inoculation (cm)**		
	Colletotrichum gloeosporioides	***Sclerotium rolfsii***	***Fusarium sp***
PDA	9	9.0	4.6
Czapek'dox	7.6	0.0	8.3
Oat meal	8.1	9.0	6.5
Maize meal	4.8	9.0	4.4
SEm (±)	**0.07**	**0.02**	**0.13**
CD (P=0.05)	**0.22**	**0.07**	**0.41**

Important cultural characteristics of these pathogens in different media were dictated in the table-5.

Table no.5: Cultural behaviour of these pathogens in different media:

Media	***Colletotrichum***	***Sclerotium rolfsii***	***Fusarium sp***

	gloeosporioides		
PDA	At first milkish white mycelia growth started on media. Acervuli formation started at the whole periphery of the plate and after some day covered the plate.	Creamy-white dense fungal colony; back of the media was cream colored.	Concentric fungal colony having pink to light brown center and black borders. The borders were again surrounded by pink outlines. They had patches of white, slightly dense colony over them.
Czapek' dox	Whitish submerged mycelia growth started. Black granulation (acervuli) are covered the whole plate but it was not so dense.	No growth was observed.	Concentric fungal colony having pink to light brown center and black borders.
Oat Meal	Whitish mycelium growth on which black dense doted acervuli are present.	Creamy-white dense fungal colony; back of the media was cream colored.	Olivaceous green centre with fluffy growth over the media.
Maize Meal	Whitish mycelia growth is formed. Black acervuli are scattered on the mycelia growth but it not so dense.	Very white fluffy growth over the media.	Whitish mycelia growth is formed but it not so dense.

Growth of pathogens in different liquid media:

The fungus was allowed to grow in four different liquid media such as Potato Dextrose, Czepek dox, Oat meal, Maize meal broth for 15 days. After this period dry weight of these pathogens was measured. The results (Table-6) revealed that highest dry weight of

Colletotrichum gloeosporioides was observed in PDA media. In case of *Sclerotium rolfsii* highest dry weight observed in maize meal media and highest dry weight of *Fusarium sp* observed in czapek dox media.

Table no. 6: Dry weight of pathogens in different media:

Media	Dry weight of pathogens		
	Colletotrichum gloeosporioides	***Sclerotium rolfsii***	***Fusarium sp***
PDA	1.47	1.32	0.61
Czapek'dox	1.19	0.1	1.34
Oat meal	1.31	0.81	0.95
Maize meal	0.73	1.48	0.59
SEm (±)	**0.01**	**0.02**	**0.03**
CD (P=0.05)	**0.05**	**0.06**	**0.09**

Micrometric measurements of the spores produced by these pathogens:

Micrometric measurement of the pathogen were made after growing in PDA media and observed under the high power microscope (Table no. 7).

Table no. 7: The length and breadth of the spores (conidia) of the isolated fungi:

Name of the Crops	Name of the Fungus	Length (µ)	Breadth (µ)
P. longum	*Colletotrichum gloeosporioides*	5.60-8.50	0.55-1.38
T. indica	*Sclerotium rolfsii*	-	-
H. subdariffa	*Fusarium sp.*	45-50	15-20

Conclusion: It is evident that early bight disease is one of the important diseases of medicinal plants due to higher disease incidence and severity resulting maximum crop damages in the untreated plots with suitable control measures. The infecting agent of leaf blight disease was different in different medicinal plants. *Colletotrichum gloeosporioides* in *P. longum, Sclerotium rolfsii* in *T. indica* and *Fusarium* sp in *H. subdariffa* were found to cause the disease. Again these pathogens also required different media for their better growth.

Future scope: Identification of these pathogens should be done on the basis of molecular techniques. Simultaneously their control measures need to be carried out for the reduction of the disease incidence in terms of better yield.

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