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Studies on important diseases of Antomul (*Tylophora indica*) and Tak Bhindi (*Hibiscus subdariffa*) with special emphasis on management of the diseases

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ABSTRACT

Among the cultivable medicinal plants in India particularly in West Bengal, Antomul (Tylophora indica) and Tak bhindi (Hibiscus subdariffa) is one of the most important crops which suffers from leaf blight disease caused by Sclerotium rolfsii and Fusarium sp. respectively. The results of fixed plot survey showed that highest blight disease of Antomul was recorded during June 2013 and lowest disease incidence was recorded during September 2012 and highest blight disease of Takbhendi was recorded during May 2013 and lowest disease incidence was recorded during August 2013. Studies of symptoms of leaf blights of Antomul and Takbhendi were recorded. The causal pathogens Sclerotium rolfsii and Fusarium sp. respectively were established through pathogenicity test. Morphometric characters and micrometric measurement of the pathogens were made. Field trial by using safer fungicides and biocontrol agents showed that application of Mancozeb @ 0.25% recorded the lowest percent disease incidence of blight of Antomul caused by Sclerotium rolfsii and application of Carbendazim @ 0.1% recorded the lowest percent disease incidence of blight of Takbhendi caused by Fusarium sp.

Key words: *Sclerotium rolfsii, Fusarium sp., Mancozeb, Carbendazim*

INTRODUCTION

India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha and traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value[4,5]. Several biotic factors like fungi, viruses, bacteria, phytoplasmas, nematodes and abiotic factors like deficiencies in soil, lack of proper irrigation, etc. are responsible for the maladies of medicinal plants. Swart and Langenhoven[6] reported *Botrytis* blight on *Hibiscus* spp. in South Africa caused by *Botrytis cinerea*. A very little work other than the work conducted under AICRP on medicinal and aromatic crops has been made on diseases Antomul (*Tylophora indica*) and Tak bhindi (*Hibiscus subdariffa*). In this present investigation attempts have been made to study the diseases of Antomul (*Tylophora indica*) and Tak bhindi (*Hibiscus subdariffa*) caused by *Sclerotium rolfsii* and *Fusarium* sp. respectively with special emphasis on management of the diseases.

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METERIALS AND METHODS

Fixed plot survey:

Observations of the plots were done at 15 days intervals starting from the month of August, 2012 to June, 2013 where the incidence and severity of the diseases which appeared on Antomul (*Tylophora indica*) and Tak bhindi (*Hibiscus subdariffa*) were recorded. For percent disease incidence total no. of leaves/stems infected in a plot were recorded and for percent disease index, no. of leaves infected per 10 plants in each plot 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. Percent disease incidence and percent disease index were calculated from the following formulae:

$$\text{Percent Disease Incidence} = \frac{\text{No. of infected leaves per plant}}{\text{Total no. of Leaves Per Plant}} \times 100$$

$$\text{Percent Disease Index} = \frac{\sum \text{Numerical Ratings}}{\text{Total no. of units observed} \times \text{maximum rating}} \times 100$$

Study of the disease symptom:

Disease conditions in the plants were recognized according to the symptoms produced by the pathogens. The plants were carefully studied and symptoms observed on the plants were recorded.

Isolation of the pathogen

Collection of disease specimen:

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 Antomul and Tak Bhindi

Method of isolation:

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 for 1 min. and were later rinsed three times with sterile distilled water under aseptic condition. With the help of a sterilized forceps, each piece was placed aseptically on the solidified PDA on the 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 sporulation and identification of the pathogen.

The isolates were maintained on potato dextrose agar medium. All the isolates were preserved at 5^0C. Sub cultures were made at 15 days intervals.

Pathogenicity test:

Pathogenicity of isolated fungi was tested on potted plants by inoculating the leaves after removing all diseased leaves. The test was conducted with 3 replications and 5 plants per replication. Suitable control was maintained by spraying water. A spore suspension (5 x 10^5 spores/ml) was prepared from 8 days old culture grown on potato dextrose agar medium was sprayed on leaves, with an all glass automizer and the whole set up was placed in the humid chamber. The lesion appeared after 2-3 days of inoculation were observed.

Confirmation of pathogens:

After the appearance of disease symptoms, 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.

Morphometric character of the pathogen:

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 and 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.

Colony characters and radial growth of *Sclerotium rolfsii* and *Fusarium sp*.**in different semisolid media:**

Sclerotium rolfsii and *Fusarium sp*. were grown in PDA, Czapek dox, Maize meal and Oat meal media. Molten medium was poured into each sterile Petriplate and allowed to solidify. Small discs (6 mm) of the fungus mycelium was cut with a sterile disc cutter from margin of 7 days old culture grown in PDA and was transferred asceptically to the plates and incubated at 28 ± 1^{o}C. Different changes of fungal colony in different media were recorded every day up to 10 days. Colony diameters were measured up to 10 days from 2nd day of inoculation.

Growth of *Sclerotium rolfsii* and *Fusarium sp*. in different liquid media:

Sclerotium rolfsii and *Fusarium sp*. were grown in PDA, Czapek dox , Maize meal and Oat meal broth i.e. liquid media (50 ml in 250 ml Erlenmeyer flasks). All the flasks were inoculated with 6 mm mycelial disc grown on potato dextrose agar medium and incubated at 28 ± 1^{o}C for 8 days. After 8 days dry weight of mycelial mats were recorded.

Dry weight determination:

Coherent mycelium was removed from liquid medium Washed thoroughly and dried on a pre weighted filter paper at 65-70°C for 48 hrs. The dried mycelial mat with filter paper was kept in a desiccators over anhydrous P_2O_5 and then weighed. Weighing was repeated till constant weight was obtained.

***In vitro* management of leaf blight disease of Antomul and Tak bhindi**

Effect of fungicides on hyphal growth of *Sclerotium rolfsii* and *Fusarium sp*.:

The fungicidal solutions were prepared on the basis of active ingredients (ai) of the products and to determine the fungicidal effect on hyphal growth, poisoned food technique was followed using PDA as food base. After autoclaving and cooling (45–50^{0}C) different concentration of fungicides as per treatment were incorporated/mixed into the molten Potato Dextrose agar media. This sterile molten media containing fungicide was poured aseptically into sterile petriplates. In control only molten media without fungicides was poured. Each treatment as well as control was replicated thrice. Each plate was aseptically inoculated with mycelial disc and incubated at 28 ± 1^{0}C. The colony diameter of the fungus was measured when in control full plate growth was observed.

Per cent inhibition of growth was calculated by the formula:

$$\% \text{ Growth Inhibition} = \frac{\text{Growth In Control} - \text{Growth In Treatment}}{\text{Growth In Control}} \times 100$$

ED_{50} values of different fungicides towards inhibition of hyphal growth was determined by log probit analysis (Log of Concentration of fungicide and probit value of hyphal growth inhibition).

Screening of antagonist against *Sclerotium rolfsii* and *Fusarium sp*.

Antagonistic potential of *Trichoderma* isolate:

The antagonistic properties of *Trichoderma* isolates which were collected from the laboratory of AICRP on Medicinal and Aromatic plants and Betelvine was tested on PDA medium by Dual Culture Plate Technique. 5 days old culture of the fungi under study were plated aseptically at the edge of petri plates 2 days before the placement of *Trichoderma sp*. Paired cultures were observed for a total of 9 days before being discarded. All the ratings were done after contacts between pathogens and antagonist using a modified Bell's[1] scale (1-5) developed as follows:

Class I (R1) – The antagonist completely overgrew the pathogen (100% overgrowth).

Class II (R2) – The antagonist overgrew at least 2/3 rd of pathogen surface (75% overgrowth).

Class III (R3) – The antagonist colonized on half the growth of the pathogen (50% overgrowth).

Class IV (R4) – The pathogen and antagonist locked at the point of contact.

Class V (R5) – The pathogen overgrew the mycoparasite.

FIELD TRIAL:

A field trail was conducted at 'C' Block farm, Kalyani. The chemicals and bioagents were sprayed at 15 days interval for three times.

To prepare a spore suspension of *Trichoderma* spp, eight days old culture plates were used, which were grown on PDA at 28±1ºC. The plates were rinsed by brush with sterilized distilled water. The suspension was then filtered by muslin cloth to separate the spores from the mycelia. The concentration was adjusted to 3.7×10^8 spores/ ml with the help of haemocytometer.

The treatments were:

Tr1 = Spraying of Blitox @0.25%
Tr2 = Spraying of Carbendazim @0.1%
Tr3 = Spraying of Mancozeb @0.25%
Tr4 = Spraying of *Trichoderma-1* @ 3.7×10^8 cfu per ml
Tr5 = Spraying of *Trichoderma*-2 @ 3.7×10^8 cfu per ml
Tr6 = Spraying of *Trichoderma*-3@ 3.7×10^8 cfu per ml
Tr7 = Control

Spraying was done as per treatment schedule. Before starting the experiment all the infected leaves in treatment rows were removed. Last application of chemicals is done in the month of July. Results were recorded by counting the number of infected and healthy leaves in treatment rows. Per cent disease incidence (PDI) was calculated as mentioned earlier. The results obtained were subject to analysis of variance.

RESULT AND DISCUSSION

Fixed Plot Survey:

The results (Table 1) revealed that the disease is present throughout the year in *Tylophora indica* and *H. subdariffa*. In *T. indica* the lowest disease index (20.00%) was recorded in the month of August, 2012 whereas the lowest disease incidence was recorded during the month of September, 2012. In case of .*H.subdariffa* both the per-cent disease incidence (10.11%) and index (15.33%) was found to be lowest during the month of August, 2012. In case of *T. indica*, both the disease incidence (35.48%) and index (72.66%) was found to be maximum during the month of June, 2013 while in case of *H.subdariffa*, the maximum disease incidence (26.48%) and disease index (67.33%) were recorded during the month of May, 2013 and June, 2013 respectively.

Symptoms

Leaf blight of *Tylophora indica*:

Symptoms first appeared on the tips of the leaves where yellow water soaked discoloration occur. Later, the margin of the leaves also started yellowing uniformly and spread gradually downwards and inwards the leaves until the entire leaf blades turned yellow and ultimately wilted and died. The main characteristic symptom was that the tips of the leaves gave a burnt appearance.

Table 1. Fixed Plot Survey of Diseases

Crops	*T. indica*		*H. subdariffa*	
Months	% Disease Index	% Disease Incidence	% Disease Index	% Disease Incidence
Aug,12	20	10.33	15.33	10.11
Sept,12	24	9.90	20	12.37
Oct,12	28.67	12.31	24.66	17.55
Nov,12	34	14.96	30.66	20.63
Dec,12	42	19.55	40	22.33
Jan,13	47.33	20.58	45.33	23.36
Feb,13	52.67	24.22	50	23.69
Mar,13	58	28.02	58	24.44
April,13	63.33	30.57	62.67	24.84
May,13	67.33	34.29	64.67	26.48
June,13	72.66	35.48	67.33	25.46

Leaf blight of *Hibiscus subdariffa*:

Dark chocolate, irregular shaped spots without any halo and gry spots were present on the leaves. Spots tend to coalesces together causing blighting of the leaves. Spots could be visible on both the surface of the leaves.

Colony Characters of the Pathogens:

The Colony Characters of the Pathogens were studied 5 days after inoculation in PDA Media by Visual Observation and under Phase – Contrast Microscope (Table 2).

Micrometric Measurements

Micrometric measurement of the pathogen were made after growing in PDA media and observed under the high power microscope (Table 2).

Table 2. Colony Characters of the Pathogens and the length and breadth of the spores

Name of the crop	Name of the fungi	Colony characters of the pathogens	Length of spore	Breadth of spore
T. indica	*Sclerotium rolfsii*	Very white fluffy growth over the leaf surfaces. Tip of the hyphae looked like it would produce conidia like structures; mycelium was septate.	1 – 2 MM	
H. subdariffa	*Fusarium sp*.	Olivaceous green center with fluffy growth over the leaf surfaces. Produced micro-conidia which were boat-shaped and bicelled.	45-50µ	15-20µ

Growth of Pathogens in Different Semi Solid Media:

The fungus was allowed to grow in four different media such as Potato Dextrose Agar media (PDA), Czepek dox media (CZA), Oat meal agar media (OMA), Maize meal agar media (MMA). Data was taken for ten days. The results (Table 3) showed that the highest growth of *Sclerotium rolfsii***,** was 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 3).

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 that dry weight was determined. The results (Table 3) revealed that in case of *Sclerotium rolfssi* highest dry weight observed in maize meal media and highest dry weight of *Fusarium* sp observed in czapek,dox media.

Antagonistic study of *Trichoderma* against pathogens:

Trichoderma strains were isolated from soil samples collected from AICRP on medicinal and aromatic plants and Betelvine.

The results (Table 4) revealed that in case of *Sclerotium rolfssi* and *Fusarium sp* where T-1 and T-2 were moderately antagonistic and T-3 was weak antagonistic.

Table 3. Colony characteristics and growth of the pathogen in different media

Media	*Sclerotium rolfsii*	*Fusarium* sp.	Radial growth in different media (cm.)		Dry wt. (gm) in different liquid media	
			S. rolfsii	*Fusarium* sp.	S.*rolfsii*	*Fusarium* sp.
PDA	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.	9.0 cm	4.6 cm	1.32 gm	0.61 gm
Czapek dox	No growth was observed.	Concentric fungal colony having pink to light brown center and black borders.	0.0 cm.	8.3 cm	0.1 gm	1.34 gm
Oat Meal	Creamy-white dense fungal colony; back of the media was cream colored.	Olivaceous green center with fluffy growth over the media.	9.0 cm	6.5 cm	0.81 gm	0.95 gm
Maize Meal	Very white fluffy growth over the media.	Whitish mycelia growth is formed but it not so dense.	9.0 cm	4.4 cm	1.48 gm	0.59 gm

Table 4. Screening of *Trichoderma* isolates against *Sclerotium rolfsii* and *Fusarium* sp.

Pathogens	Isolate of *Trichoderma* sp.	Point of contact (day)	Bell's ranking
*Sclerotium rolfs*si	T-1	3	R2
	T-2	3	R2
	T-3	3	R3
Fusarium sp.	T-1	3	R2
	T-2	3	R2
	T-3	3	R3

Bioassay of pathogens against some chemicals:

Three fungicides ie. Blitox, Bavistin and Mancozeb were tested against *Sclerotium rolfsii* and *Fusarium sp* by poisoned food technique and the result presented in Table 5. The result revealed that highest ED_{50} value was recorded in case of Carbendazim against *S.rolfsii* and *Fusarium sp.* Lowest ED50 value was noticed where Copper oxychloride were tested against *S. rolfsii* and *Fusarium* sp.

Table 5. ED_{50} value of different fungicides towards mycelial growth of *Sclerotium rolfsii* and *Fusarium* sp.

Name of the fungicide	Trade name	Chemical name	ED_{50} value in ppm	
			S. rolfsii	*Fusarium sp.*
Copper oxychloride (50WP)	Blitox	Copper oxychloride preparation	275.42	112.2
Carbendazim	Bavistin	Methyl-2-benzimidazole carbamate	316.22	446.68
Mancozeb	Dithane M-45	Manganous ethylene bisdithiocarbamate	<50	398.1

***In vivo* management of diseases of Pipul (*Piper longum*), Antomul (*Tylophora indica*) and Tak bhindi (*Hibiscus subdariffa*) by using safer fungicide and bio control agents**

Management of leaf blight disease of *Tylophora indica*:

Lowest disease (21.48%) was recorded in treatment where spraying of mancozeb (0.25%) were made (T3) and it was statistically at par with all the treatments except where spraying of *Trichoderma*-1(T4), spraying of *Trichoderma*-2 and where no spraying (control treatment) were made. Highest disease (53.75%) was recorded in control treatment (Table 6).

Management of leaf blight disease of *Hibiscus subdariffa*:

The result(Table 6) revealed that the lowest disease (23.04) was recorded in treatment where spraying of carbendazim (0.1%) were made (T2) and it was statistically at par with all the treatments except in treatments T1, T3 and T6 where blitox (0.25%), mancozeb (0.25%) and *Trichoderma*-3 were sprayed respectively. Highest disease (55.41%) was recorded in control treatment.

Table 6. Management of leaf blight of *Tylophora indica* and *Hibiscus subdariffa*:

Treatments	Precent disease incidence		Percent disease control	
	Leaf blight of *Tylophora indica*	Leaf blight of *Hibiscus subdariffa*	Leaf blight of *Tylophora indica*	Leaf blight of *Hibiscus subdariffa*
Spraying of Blitox (0.25%)	14.19 (22.77)[1]	16.46 (23.94)	57.64	56.79
Spraying of Carbendazim (0.1%)	20.37 (26.81)	9.18 (17.63)	50.12	68.18
Spraying of Mancozeb (0.25%)	13.42 (21.48)	19.16 (25.44)	60.04	53.19
Spraying of *Trichoderma*-1	34.72 (36.08)	25.44 (30.27)	32.87	45.37
Spraying of *Trichoderma*-2	47.57 (43.59)	31.09 (33.87)	18.9	38.87
Spraying of *Trichoderma*-3	24.39 (29.58)	19.99 (26.54)	44.97	52.1
Control	73.13 (53.75)	67.81 (55.41)		
SEm±	5.03	4.57		
CD at 5%	11.4	12.68		

[1] Figures in parentheses are the angular transformed values of percent disease incidence

The results thus obtained are in consonance with the results obtained by Chowdhury *et al*., 2011, 2015 where application of Carbendazim and Mancozeb recorded lowest disease incidence for the management leaf spot of Thankuni and target leaf spot of Sarpagandha in comparison with the biocontrol agents although the results obtained were at par with the spraying of *Trichoderma* -3. However, for the management of any medicinal plant diseases, it will be better if we can control the disease by using biocontrol agents to reduce the toxic hazards of fungicides on human beings. So, more nos. of biocontrol agents should be tried for the management of diseases of medicinal plants. The results thus obtained needs further investigation before being recommendation to the farmers.

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